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ANALYSIS OF ERRORS IN ELECTRONIC BLOOD CELL COUNTING

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Abstract. The present work is based on the use of purified blood cells in analysing possible errors in electronic counting. In counting of erythrocytes, no great errors occurred. In counting of platelets a primary diluting fluid prepared from Isopaque® was found useful to obtain constant separation and to avoid correction for hematocrit. In counting of leucocytes the composition of the diluting fluid was essential, saponin caused complete hemolysis and reduced the interference from platelets. A new method was used for calibrating the electronic particle counter which simplified the technique and reduced the interference from other cells.

The electronic particle counter has largely replaced microscopic counting of erythrocytes (E) from peripheral blood, but has not become so widely accepted for counting of platelets (P) and leucocytes (L).

The electronic counting of P is dependent on the efficiency with which P are separated from E and L, either at standstill (4, 9, 15, 17) or by centrifugation (2, 5, 6, 7, 10, 12). The correction for hematocrit needed in most methods is cumbersome.

The electronic counting of L is highly dependent on whether the hemolysis is complete, since the ratio L/E is so unfavourable (~1/1 000). It is not yet settled whether saponin (16), cetrimide (11, 13) or Triton-X 100 (1, 8, 12) is the preferable substance.

Additional problems in electronic counting of blood cells are the different composition of diluting fluids, the coincident loss (14) and the interference from other cells in counting, a problem not so far studied.

This work introduces purified cell fractions in analysing problems of electronic counting of blood cells in the attempt to evaluate appropriate techniques.

MATERIAL AND METHODS

Electronic particle counter

A Celloscope 101 (AB Lars Ljungberg, Sweden) was used. This apparatus has two capillaries, 0 and 1, with 50 and 70 μ aperture diameter respectively, a threshold setting (discriminator-*D*) which excludes particles below a certain size from being counted (scale 0–100), and an electric current regulator (*K*) which determines the height of oscillations (scale 0–100). The following settings were recommended by the manufacturer: capillary 0 and *D* 10, capillary 1 and *D* 20, capillary 1 and *D* 40 for counting of P, E and L respectively. The procedures used in counting the different blood corpuscles from peripheral blood are shown in Fig. 1.

Purified cell fractions were separated from peripheral blood of healthy blood donors as described elsewhere (19).

Analysing methods

The reproducibility in counting was determined by the coefficient of variation of replicate counts.

The stability of cell suspensions was measured by repeated counting with the Celloscope, decrease in cell concentration being due to irreversible aggregation (18). This method was used to evaluate suitable diluting fluids and to judge which hemolytic substance is preferable: saponin (BDH, England), Cetavlon (ICI, England) or Triton-X 100 (Sigma, USA).

The coincident loss was measured by comparing the theoretical cell concentrations obtained through dilutions with results measured with the Celloscope.

The interference was measured as the percentage of one cell fraction which was counted as another. The interference at different settings of the Celloscope was judged from cumulative frequency distribution curves of purified cell suspensions.

The problem of obtaining constant separation of P was studied by measuring sedimentation of P exposed to centrifugation. A clear zone at the top suggested inappropriate technique; decrease in P/μl in the upper zone, or decreased recovery of P measured with the Celloscope, was used as a more exact measurement of sedimentation. Primary diluting fluids were used to obtain the desired density of P-rich suspensions or blood, Isopaque® (Nye-

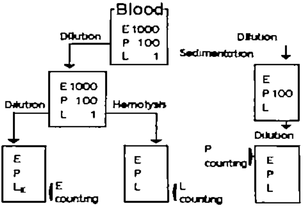


Fig. 1. Procedures for counting the different blood corpuscles with an electronic particle counter. (P and L_E), (E and P_L) (E_P and L_P) symbolize the interference in counting of E, L and P respectively.

gaard, Norway) (Natrii-*N*-methyl-3,5-diacetamido-2,4,6-triiodobenzoate) 440 mg iodine/ml, e.g. primary diluting fluid with density 1.05 is calculated from the equation:

x1.421 + 0.02 1.05 + (1-(0.02 + x)) 1.003 = 1.05 1
Isopaque EDTA 10% saline 0.9%

Microscope counting was used as reference in calibrating the Celloscope. In counting of E or P phase contrast microscope, Bürker counting chamber and the diluting fluid D in Table I were used. The Bürker chamber was kept in moist chamber for 15 min before counting of P.

Table I. *The stability of leucocyte counts with different diluting fluids*

Each number is the mean of replicate counts from four samples

Reagent	Diluting fluid (ml) A	B	C	D	E
NaCl 0.9%	1 000	990	940	930	970
EDTA 1%		10	10	10	
Phosph. buffer M/15			50	50	
Formalin 35%				10	10
Acid. acet. glac.					5
Cetavlon 40 ..					12.5
Saponin added	+	+	+	+	−
Time after hemolysis, min	Leucocyte counts (%)				
0	95.4	93.5	99.0	100.0*	
1/2	81.0	86.5	94.5		See
10	43.2	37.0	94.5	101.9	text
30	18.3	33.3	85.5	100.8	
S.E.	3.1	2.3	1.8	1.0	

* Reference count.

Calculations

Instead of using the "right R" which makes cells/μl determined with the Celloscope (C_E, C_L, C_P) and microscope (M_E, M_L, M_P) equivalent, the author preferred to use an appropriate R'' (see later) and constant multiplying factor:

$$M_E = C_E\, f_E = (x_E - x_0)\ \text{Dilution}\ f_E/8 \quad \sim x_E\, f_E\, 10^4 \quad (1)$$

$$M_L = C_L\, f_L = (x_L - x_0)\ \text{Dilution}\ f_L/8 \quad \sim x_L\, f_L\, 10^2 \quad (2)$$

$$M_P = C_P\, f_P' = (x_P - x_0)\ \text{Dilution}\ f_P f_{Hct}/8 \sim x_P\, f_P\, 10^3 \quad (3)$$

Cells/μl are obtained by dividing by 8 since each 64th particle in 512 μl diluting fluid is registered on the decade counter of Celloscope 101. X_0 is the control value,

$$f_{Hct} = 1 - \frac{\text{Hct/Dilution}}{100}$$

is due to correction for hematocrit using primary Dilution (times diluted). The error introduced in per cent, if no correction is made for hematocrit, should be:

$$\frac{(\text{Hct}_0 - \text{Hct})\ 100}{100\ \text{Dilution} - \text{Hct}_0}$$

where Hct_0 is the mean hematocrit at calibration and Hct is the actual hematocrit.

Calculation of interference: $E + (P_E + L_E) = 100$, where $(P_E + L_E)$ means the percentage of total count which is due to interference from P and L in counting of E.

RESULTS

The reproducibility of replicate counts was found to be 0.71, 0.71 and 1.31 for E, P and L respectively (20 samples in each group).

Coincident loss was found to be small, except at relatively high cell concentrations (Fig. 2); the deviation occurred at equal rates of counting for E and P (~1 500 particles/sec).

The following procedure may be advisable for choice of the appropriate R-values. In counting of E an appropriate R'' should be chosen at

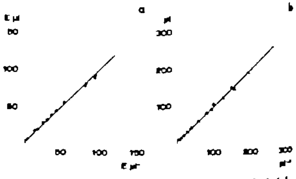


Fig. 2 (*a* and *b*). Relation between the theoretical (*abscissa*) and estimated (*ordinate*) cell concentrations. Actual counts obtained by multiplying by 8. Each point is the mean of replicate counts of 4 samples.

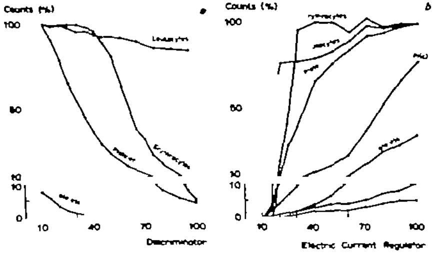


Fig 3 (*a*) The percentages of E, L and P which are counted as E at "right R" (R 46) and different D (*b*) The percentages of P E and L which were counted at different *R*. The control values E( ), P(*c*) and L(*c*) are given in absolute counts. Platelets (E) show the percentage of P which was counted as E.

some distance from the incipient plateau to include different size of E occurring in disease, but not so high that P interferes too much (Fig. 3 *b*). At such an appropriate R'' ($R = 46$, in this case equal to the right R''), the interference was found to range between 2 and 8% of P and nearly 100% of L (Fig. 3 *a*) at normal cell ratios (Fig. 1) this contributes to a total interference of 0.8% In counting of P an appropriate R'' should be chosen to include most P (Fig. 3 *b*), but not so high that noise (control values) interferes too much. In counting of L, ghosts of E" did not interfere using saponin as hemolytic substance: the control values were not exceeded although five times the concentration of E were hemolysed and counted at increasing *R* values. Fig. 4 *a* shows that P might interfere with the counting of L. clouds" of free P did interfere more than aggregates of P caused by addition of saponin, lysis of P was excluded by examination in a microscope. The interference from aggregates of P increased with the concentration of P and *R*-values (Fig. 4 *b*). The shape of the cumulative frequency distribution curves of L in a hemolysed blood sample (Fig. 4 *b*) differed greatly from the similar curve of purified L (Fig. 3 *b*) most likely due to interference from P An appropriate R'' value in counting of L should be chosen at the incipient plateau of the L-curve (Fig. 3 *b*), at which the interference from P is the smallest possible.

The composition of the diluting fluid was found to be particularly essential in counting of L. With the diluting fluid E in Table I, a great dispersion of results occurred. The diluting fluid D in Table I was found advisable for counting of all blood

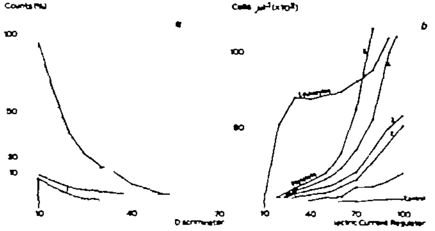


Fig 4 (*a*) P counted as L before (I) and after (II) addition of saponin at different D curves 1 and 2 are the corresponding corrected curves showing the percentage interference of P in counting of P counted as L. (*b*) The of L (in cells/μl) using saponin; curves 1 2, 3, 4 5 correspond to 1 3 5 10 and 20 10^4 P/μl of blood. The "Leucocytes" curve is a typical curve showing how L/μl depend on the *R* value.

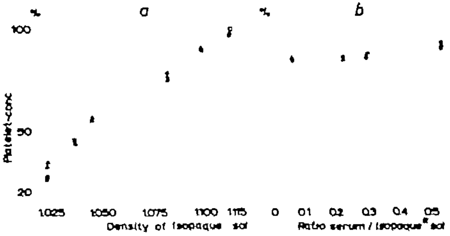


Fig 5 Sedimentation of P examined in relation to different density of the primary diluting fluid (*a*), and different quantity of serum (*b*). Equal parts of serum and Isopaque solution were used in (*a*), and in (*b*) the density of the Isopaque serum was kept constant (1.08).

corpuscles but formalin should preferably be reduced to 2 ml, otherwise hemolysis might be delayed. Saponin was found to be the preferable hemolytic substance, since the hemolysis was complete and high stability in counting was obtained.

The problem of obtaining constant separation of P was examined in relation to different diluting fluids prepared from sucrose, glucose, EDTA, glycerol or Isopaque® of which Isopaque was found preferable. The sedimentation of P was reduced by increasing the density of the primary diluting fluid (Fig. 5 *a*) and by avoiding high primary dilutions (Fig. 5 *b*). The following primary diluting fluid was found advisable: Isopaque 20 ml, EDTA (10%) 1 6 ml, saline (0.9%) 60 ml. The error introduced, if no correction was made for hematocrit, is small using a primary dilution of 1 11 (1.5% at Hct = 30 instead of $Hct_{e} = 45$) The constant multiplication factor of P (f_P') was independent within the range of primary dilution used, but was highly related to the settings of the instrument (Table II). The admixture of E in the P-rich supernatant after centrifugation of blood/Isopaque solution (density 1.08) at dilution 1 1 1 6 and 1 11 was found to be 0 02, 1 0 and 4 0% respectively measured with a microscope the differences ($p < 0.001$ $N = 10$) were possibly due to osmotic influence on E.

Table II. *Calculation of f′ at different primary dilutions of blood and Celloscope settings*

The density of Isopaque solution given in parentheses. Each experiment consists of replicate counts of four samples with the Celloscope and of counting at least 1000 P with the microscope. f_p' is calculated from equation (3).

Blood/Isopaque sol.	No. of exp	Celloscope settings Cap	D	R	f
1/1 (1.08)	1	0	10	46	1.30
1/6 (1 10)	3	0	10	46	1 33
1/10 (1 10)	3	0	10	46	1.27
1/6 (1 10)	3	0	10	70	1.05
1/10 (1.10)	3	0	10	70	1.02
1/6 (1.10)	3	1	10	80	2 41
1/10 (1 10)	3	1	10	80	2.49

DISCUSSION

The high reproducibility of electronic particle counters (3) makes it desirable to replace the tiresome method of microscope counting if other problems in electronic counting can be satisfactorily solved.

The composition of the diluting fluid was found to be particularly essential in counting of L due to the aggregating influence of hemolytic substances. The difficulties which have been ascribed to the different quality of saponin (8) may as well have been due to the diluting fluid.

The coincident loss is reduced at an appropriate setting of the instrument, at which the resolution capacity is high, by correcting all counts according to a table (14) by reducing the range of counting by using different dilutions, or most simply by avoiding high rates of counting.

A mean correction of interference is included in the calibration of the electronic particle counter it is the change of cell ratios from that at calibration which introduces errors. The interference in counting of E from L was negligible,

except in leukemia where L have to be subtracted from E. In counting of L no interference was found from "ghosts" of E using saponin as hemolytic substance; this uncertainty (8) therefore seems to have been resolved. P are not thought to interfere in counting of L. However both free P and aggregates of P did interfere; this was reduced to a minimum at an appropriate *R*. In counting of P it has been suggested that the admixture of E measured with an electronic particle counter should be subtracted; this was found to give false values due to interference from P, for which reason it is advisable to use a method in which the admixture of E is negligible.

In counting of P the problem of obtaining constant separation is theoretically solved by increasing the density of plasma above that of P. However the substance added to plasma may complicate the separation due to loss of protection from plasma proteins (20). This possibly explains the increased sedimentation of P at high primary dilutions. Isopaque seems so far to be the preferable substance due to small osmotic activity.

The new method of calibrating the electronic particle counter may at first seem inconvenient, but it reduces the interference and simplifies the technique. Since the diluting constant is included in the constant multiplication factor, the diluting technique may be simplified as it needs only one automatic pipette. A further simplification might be obtained by applying the rate principle instead of the constant volume principle: cells/μl ($_0\int Y$ dt) are obtained by adjusting the time (t). Another advantage of using the rate principle is that it simplifies and improves the registration of cumulative frequency distribution curves and Price Jones curves.

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THE MORPHOLOGY OF THE ERYTHROCYTE SEDIMENTATION RATE (ESR)

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Abstract. Erythrocytes in normal autologous plasma formed rouleaux, which increased in speed and degree with the cell concentration, forming fine-meshed net- of rouleaux at high cell concentrations. In cases of logically or artificially induced elevation of the sedi- on rate, a qualitative change of the red cell inter tion has been found, characterized by the tendency to clumping of rouleaux with coarse-meshed network of rouleaux between such clumps.

The erythrocyte sedimentation rate (ESR) was introduced by Fåhraeus about 50 years ago (2). Yet the mechanism of ESR is still not well understood (7) and particularly the reason why the normal ESR is lower than expected according to physical laws. The normal ESR is thought to be due to a sedimentation of separate cells, whereas the elevated ESR should be caused by rouleaux (11). The rouleaux phenomenon, known for about 200 years (10), is closely related to the biconcave shape of the erythrocytes, which presumably depends on the composition and architecture of the erythrocyte membrane (5). There are probably many factors in plasma which may interfere with rouleaux formation, one of which, the influence of fibrinogen, is well established (3). Different substances which increase the ESR have been used *in separating techniques* (8).

The actual behaviour of the red cells during sedimentation is not so easily observed by the usual measurements. In the present work diluted samples of blood were used in order to study the morphology at different cell concentrations.

MATERIAL AND METHODS

Blood samples were obtained from healthy blood donors and from patients with elevated ESR. The following plasma proteins were determined: fibrinogen (1), haptoglobin (9) and immunoglobulins (4).

Preparation technique. Blood was mixed with anticoagulant in the proportion 4:1 as is common in ESR techniques. As anticoagulant was used citrate 3.1% or heparin (Apothekernes Laboratorium, Norway) 10 I.U./ml of blood. Platelet-poor plasma was used for dilution and was prepared by high speed centrifugation.

Aggregating substances. Human fibrinogen (Kabi, Sweden), dextrans with Mw 40 000, 150 000, 250 000 and 500 000 (Pharmacia, Sweden), polyvinylpyrrolidone (Fluka AG, Switzerland), Methocel® (Dow Chemical Company, USA) and phytohemagglutinin-M solution (Difco, USA).

Morphological examination. The suspensions of erythrocytes were placed in Bürker counting chambers, sealed with immersion oil to avoid drying and streaming, and then examined by phase contrast microscopy and photography; the fields were chosen arbitrarily. The cell concentration was determined by counting with an electronic particle counter (6).

Erythrocyte sedimentation was determined at 5-minute intervals using Westergren pipettes; the ESR curves show the sedimentation (in mm) at any time.

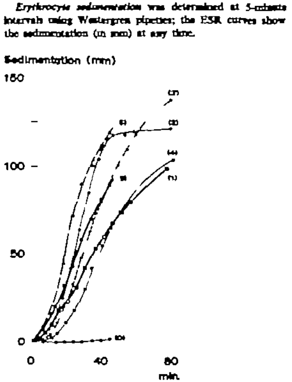


Fig. 1. ESR curves in normal blood and in cases of elevated ESR. The numbers correspond to those in Table I.

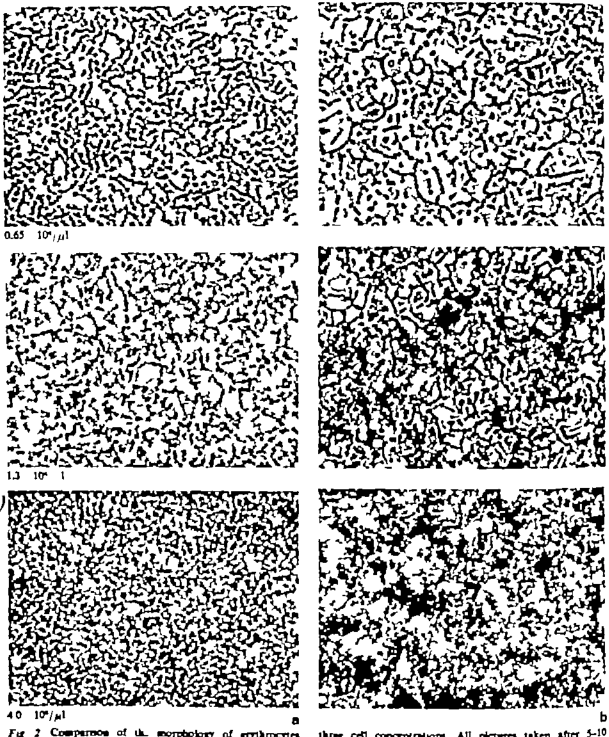


Fig. 2. Comparison of th. morphology of erythrocytes in plasma from (*a*) normal ESR and (*b*) elevated ESR at three cell concentrations. All pictures taken after 5-10 min (425)

RESULTS

Fig. 1 shows the ESR curves and Table I the corresponding plasma proteins in cases of normal and elevated ESR.

The rouleaux formation in normal plasma increased with time and cell concentration (Fig. 2 a) forming a fine meshed network of rouleaux at high cell concentrations. In cases of elevated ESR the network of rouleaux was more coarse-meshed than in normal plasma (Fig. 2 *b*) with

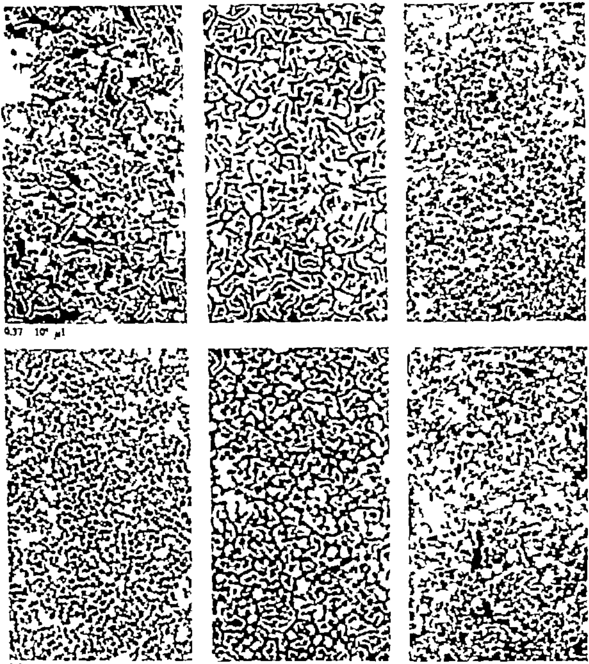


Fig. 3 Comparison of the morphology of erythrocytes in plasma from (*a*) normal ESR, (*b*) with addition of dextran Mw 250 000 (1%), and (*c*) with phytohemagglutinin-M solution (10%) at two cell concentrations. All pictures taken after 10 min (× 425).

a tendency to clumping of the rouleaux at high cell concentrations. The morphology of red cell interaction in different samples with elevated ESR did not differ very much, although the tendency to clumping differed slightly. The changes caused by fibrinogen, dextrans with Mw 150 000 250 000 and 500 000 polyvinylpyrrolidone and Methocel® (Fig. 3) resembled the morphology seen in blood from patients with elevated ESR. Dextran with Mw 40 000 did not change the

Table I. *Plasma protein pattern in cases of normal and elevated ESR*

Case no.	Plasma protein pattern (g/100 ml) Fibrinogen	Haptoglobin	γG	γA	γM	Remarks
0	0.25	0.14	1.3	0.5	0.06	Normal control
1	1.10	0.28	1.4	0.23	0.14	Wegener's granulomatosis
2	0.45	0.05	6.6	0.63	0.25	Hepatitis chronica
3	0.56	0.28	1.2	0.32	0.11	Mb. Crohn
4	0.70	0.27	1.5	0.43	0.13	Collagenosis
5	0.86	0.29	1.8	0.3	0.16	Polymyalgia rheumatica
6	0.94	0.16	2.1	1.2	0.09	Takayashu' disease
	0.2–0.4	0.03–0.18	0.75–1.9	0.05–0.35	0.04–0.28	Normal range values

morphology in normal blood. Phytohemagglutinin differed from the other substances by diminishing the rouleaux formation and by causing aggregation resembling agglutination (Fig. 3).

DISCUSSION

The different methods for measuring aggregation of erythrocytes have been dealt with elsewhere (7). Since the morphology depends on the cell concentration, aggregating time and streaming, it is essential to standardize the conditions in comparative measurements. It is assumed that the morphology described might illustrate what happens during the initial stages of sedimentation.

It was found that a fine-meshed network of rouleaux is readily formed in normal autologous plasma this network probably explains why the normal ESR is lower than one would expect (7).

In patients with elevated ESR or at artificially induced high ESR the normal fine-meshed network of rouleaux is disturbed. A close relationship probably exists between this change in morphology and the aggregating capacity present in the fluid (7) The increased speed and degree of rouleaux formation occurring in plasma from patients with elevated ESR or at artificially induced high ESR suggest that adhesions between erythrocytes are enhanced. The formation of "clumps of rouleaux" suggests that also adhesions between rouleaux are enhanced. Since most erythrocytes become included into such clumps, there are relatively few adhesions between clumps. The rouleaux and clumps of rouleaux are reversible except at high concentrations of an aggregating substance and when using phytohemagglutinin (7) In the presence of irreversible aggregation the adhesions between clumps of rouleaux delay or inhibit the ESR (7)

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THE MECHANISM OF THE ERYTHROCYTE SEDIMENTATION RATE (ESR)

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Abstract. The relation between the erythrocyte sedimentation rate (ESR) and aggregation has been studied by applying different aggregating substances. In normal autologous plasma the ESR increased with the cell concentration due to increased rouleaux formation reaching a maximum size of ESR. At high cell concentrations the ESR was retarded, probably due to formation of a network of rouleaux, which may explain the remarkably small normal ESR. The elevated ESR and the shape of the ESR curves were found to depend on the aggregating capacity present in the fluid, which determines the formation of "clumps of rouleaux" and adhesions between such clumps.

The sedimentation rate (V) of a spherical particle depends on the difference in density between the particle (S) and the fluid (S_1), the viscosity (η) of the fluid and the size (r = radius) of the particle (Stoke's law: $V = 2g(S - S_1)r^2/9\eta$). The sedimentation of erythrocytes in blood differs from the ideal model of this law due to their shape and high concentration. The normal ESR is generally assumed to be due to the sedimentation of separate particles, while the elevated ESR has been related to rouleaux formation (2, 5, 15, 20, 23). The relation between ESR and rouleaux formation in blood is difficult to interpret, since so many factors in plasma may interfere, for which reason it is preferable to study the relation between ESR and known concentrations of a single aggregating substance.

In a previous work the morphological basis of ESR was examined (18). The present work correlates the ESR and the aggregation caused by different substances. Methods for measuring the aggregation of platelets (1, 9) were not found useful for this purpose, and therefore methods for measuring the aggregation of erythrocytes had to be evaluated.

MATERIAL

Blood was obtained from healthy blood donors; heparin (Apothekernes Laboratorium, Norway) was used as an anticoagulant. In studying aggregation it was preferable to use purified cell fractions. Erythrocytes, platelets and leucocytes were separated from healthy blood donors as described elsewhere (19).

The aggregating substances tested were: dextrans (D) with Mw 40 000, 150 000, 250 000 and 500 000 (Pharmacia, Sweden), polyvinylpyrrolidone (PVP) (Fluka AG, Switzerland), Methocel® (Dow Chemical Company, USA) and phytohemagglutinin-M solution (Difco, USA).

Cell suspending media. Stock solutions were used to prepare suspending media of equal density to plasma: Isopaque® (Nyegaard, Norway) (Natrii-N-methyl-3,5-diacetamido,2,4,6-triiodobenzoase) 440 mg iodine/ml. For example, the preparation of Isopaque® solution with equal density to plasma and with 1% D 150:

$x \cdot 1.421 + 0.02 \cdot 1.05 + 0.1 \cdot 1.03 +$
Isopaque® EDTA (10%) D 150 (1%)

$(1-(x+0.12))0.998 = 1.026$
Distilled water

METHODS

Assay methods for aggregation

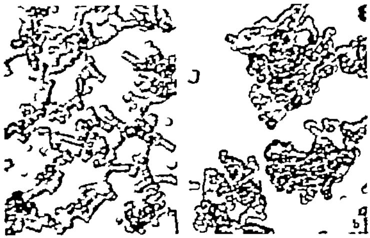
Aggregation is defined as including all kinds of cell adhesions including the rouleaux phenomenon.

Phase contrast microscopy was used to measure the percentage of erythrocytes adhering to each other in Bürker counting chambers; the chambers were sealed with immersion oil to avoid drying and streaming.

Microspectrophotometry (MPV, Leitz, Germany) was used to measure aggregation as change in transmission through suspensions of erythrocytes in Bürker counting chambers at a wavelength of 650 nm, measuring area of 1 A-square and a magnification of × 35.

An electronic particle counter (Celloscope 101, AB Lars Ljungberg, Sweden) (17) was used to measure irreversible aggregation. The cell suspensions were carefully mixed before samples were drawn; decrease in cell concentration is due to irreversible aggregation.

Reversible aggregation was measured in the Celloscope as increase in "big particles". Capillary 0 and I with 50 and 70 μm aperture diameter and a threshold setting



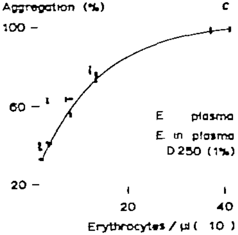


Fig. 1. The percentage of erythrocytes included in rouleaux in normal plasma (*a*), and with addition of dextran Mw 250 000 (1%) (*b*); the measurements were done in Bürker chambers after 10 min; (*c*) shows the percentage of cells adhering at different cell concentrations.

of 20 and 50 were used for platelets and erythrocytes respectively. The aperture current regulator was set to obtain an appropriate frequency of counts at the start, the measurements were thus relative. The method could be used only to measure reversible aggregation, since irreversible aggregates blocked the capillary. The reversibility was tested by mixing the cell suspension at the end of the measurements. The following diluting fluid was used: EDTA (1%) 10 ml, sodium phosphate buffer (pH 7.4) 50 ml and saline (0.9%) to 1 000 ml.

Assay methods for sedimentation

The sedimentation of erythrocytes in undiluted blood samples was measured with the commonly used Westergren pipettes; in diluted blood samples the sedimentation was measured also with spectrophotometer (Sp 500, Instruments Ltd., England). The cuvette was placed as near the photocell as possible to reduce scattering. A slit space of 10 mm height and wavelength of 650 nm were used to avoid absorption to hemoglobin. The sedimentation is measured in mm with the Westergren technique and as change in the extinction (E) with spectrophotometer using the time at "half sedimentation" (E_{50}) in comparative measurements. The ESR curves show the relation between sedimentation and time.

RESULTS

Aggregation

The percentage of erythrocytes in plasma included in rouleaux increased with the cell concentration (Fig. 1). The aggregating substances increased the speed and quantity of rouleaux formation; this was particularly evident at small cell concentration and at short aggregating time.

The change in transmission measured with MPV occurred within less than 1 min (Fig. 2*a*), suggesting that the rouleaux formation was rapid in undiluted blood samples. This method could be used to compare the relative aggregating influence of different substances (Fig. 2*b*).

The reversible aggregation measured as "big particles" with the Celloscope was found to increase with the cell concentration and time (Fig. 3). This method could be used to compare different aggregating substances at constant cell concentration and aggregating time (Table I). The method was particularly useful for measuring the reversible aggregation of erythrocytes, possibly due to the great tendency to rouleaux formation.

Irreversible aggregation of platelets occurred before that of the other blood corpuscles at high concentrations of dextran, Methocel[2] or phytohemagglutinin (Fig. 4). The degree of aggrega-

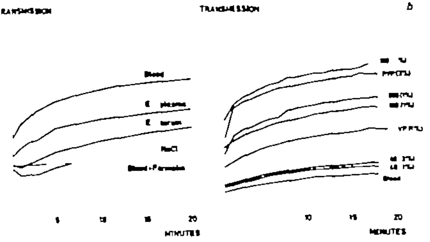


Fig. 2. Aggregation measured as change in transmission with MFV through blood samples in Bürker counting chambers. The sensitivity of the photomultiplier was increased in (*a*) compared with (*b*).

tion of leucocytes was intermediate between erythrocytes and platelets.

Sedimentation

The ESR in Isopaque® or sucrose solutions with equal density to plasma was slow, somewhat higher in serum (Table II). The aggregating substances increased the ESR more in serum than in Isopaque® or sucrose solutions (Table II). The ESR in autologous normal plasma was increased compared to serum, an U-shaped relation was found between ESR and cell concentration (Fig. 5 *a* and 6). The ESR increased towards a maximum, above which further addition of an aggregating substance did not increase the ESR (Fig. 5 *b*). The ESR was retarded at simultaneously high dextran and cell-concentrations (Fig. 6 *b* and 6 *c*): at small quantity of an anticoagulant there was tendency to delay in start of sedimentation (Fig. 6 *c*).

DISCUSSION

The explanation of the surprisingly small normal ESR can only be given indirectly. The slight tendency to rouleaux formation at small cell con-

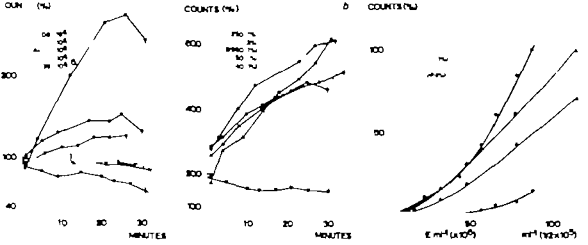


Fig. 3. Reversible aggregation measured with the Celloscope as increase in "big particles" with time: (*a*) at different concentrations of erythrocytes in diluting fluid with dextran with Mw 150 000 (1%): (b) with different aggregating substances and constant concentration of erythrocytes (the stippled lines show the reversibility on mixing): ( ) "big particles" of erythrocytes and platelets measured after 10 min with and without 1% dextran Mw 150 000.

Table I. *Aggregation measured as "big particles" with Cellascope*

Medium	Erythrocytes (4 10⁴ µl⁻¹)	Platelets (1 1 10⁴ µl⁻¹)
Control	100	100
D 40 (2 %)	265	158
D 150 (1 %)	893	104
D 250 (1 %)	1 014	126
D 500 (1 %)	1 172	131
PVP (1 %)	686	123
Fibrinogen (1 10)	163	121
Allowance (p ~ 0.05)	85	18
(p ~ 0 01)	104	

Mean values of replicate counts of four samples are given as percentages of control. The measurement was done after 10 min.

centration may explain why the normal ESR is thought to be due to sedimentation of separate cells. There seems to be a close relation between the rouleaux formation and ESR both in normal plasma, serum and Isopaque® or sucrose solutions. However at high cell concentrations a rouleaux network is easily formed (18), which may retard the ESR. The increase of ESR in anemia and the decrease in polycytemia may be related to the compactness of this rouleaux network.

The explanation of the elevated ESR and the different shapes of ESR curves might be as follows. Theoretically a linear relationship exists between sedimentation and time at constant size of and no adhesion between particles (Stoke s law). There is probably no difference between the reversible "big particles" and the clumps of rouleaux" which were seen to form in plasma with elevated or artificially induced ESR (18). A close relationship seems to exist between the ESR

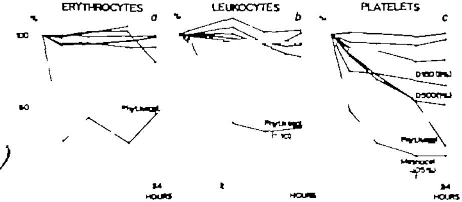


Fig. 4 Irreversible aggregation of erythrocytes, leucocytes and platelets in plasma exposed to different aggregating substances. Only curves deviating from controls (broad lines) are texted.

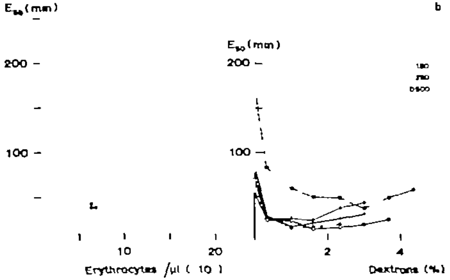
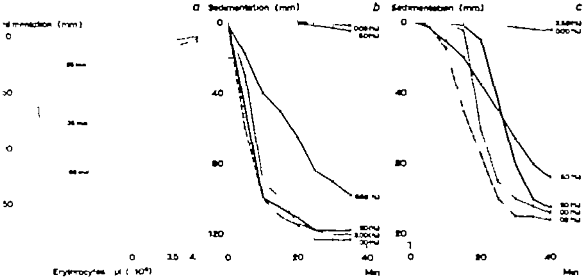


Fig 5 The ESR measured in normal autologous plasma with a spectrophotometer; (*a*) shows the time at "half sedimentation (E_{50}) in relation to the concentration of erythrocytes, and (*b*) in relation to the concentration of dextran.



ig. 6. The sedimentation measured with Westergren pipettes (*a*) the sedimentation after 25 35 and 60 min at different cell concentrations (the shaded areas are due indefinite readings), (*b* and) ESR curves of undiluted blood with addition of different quantities of dextran Mw 250 000. Heparin 10 and 3 I.U. ml of blood were used in ( ) and (*b*) respectively

and the aggregating capacity present in fluid. At small concentrations of an aggregating substance the ESR curves may have a ⌒-shape, possibly because the capacity to form "big particles is small and needs some time. At intermediate concentrations f an aggregating substance, causing maximum influence on ESR, the ESR curve approaches the linear shape. At higher aggregating capacity a marked ⌒-shape of ESR curves may occur The delay in start of sedimentation, called the aggregating period, has been thought to be due to a time lag for the aggregating substance to exercise its influence (3). This may be the ex planation at small aggregating capacity while the prolonged aggregating period at relatively high aggregating capacity is most likely due to an increase in the strength of adhesions between clumps of rouleaux, suddenly the adhesions might fail, explaining the following short sedimenting period. Theoretically these adhesions might inhibit the sedimentation entirely this may be the explanation in the case of phytohemagglutinin or of high concentrations of dextrans due to irreversible aggregation.

The phenomena occurring during sedimentation explain the efficiency in separating techniques of leucocytes from peripheral blood (19). The preferred aggregating substance should increase the ESR without causing aggregation of platelets or leucocytes. The complete delay in start of sedimentation, which might occur at high concentrations of an aggregating substance, at small quantities of an anticoagulant, and at low temperatures during sedimentation, should be avoided (19). The present study confirms the usefulness of dex trans with Mw 150 000 or 250 000 (0.8–1.0 %) (4 8, 11 13 16) polyvinylpyrrolidone (~2 %)

Table II. *The ESR in relation to different aggregating substances in media with equal density*

The ESR was measured with spectrophotometer in min at E_{∞}. ∞ = no measurable ESR within 2 h. Figures in parentheses = percentages of aggregating substance.

Aggregating substance	Isopaque® solution	Sucrose solution	Serum
0	∞	∞	135
Dextran			118 (0.3)
Mw 40 000	∞ (1.0)	∞ (1.0)	108 (1.0)
Mw 150 000	∞ (0.3)	∞ (0 3)	28 (0.3)
	24 (1.0)	65 (1.0)	23 (1.0)
Mw 500 000	25 (0.3)		30 (0.3)
	25 (1.0)	58 (1.0)	20 (1.0)
Polyvinyl-	∞ (0.7)		22 (1.0)
pyrrolidone	30 (2.0)	27 (2.0)	
Methocel®	27 (0 05)	29 (0.14)	23 (0.05)
Phyt. h. ml.	∞ (10)	∞ (10)	∞ (10)
Erythrocytes/μl	8.0.10⁶	8.0.10⁶	6.2.10⁶

(6 7 10, 16, 21 22) and fibrinogen (~0.5%) (14 16). Methylcellulose has been successfully used in flotation methods, because irreversible aggregation does not matter in this case (3 19). Phytohemagglutinin did not fulfil the demands desired in an aggregating substance (12).

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BLOOD LACTATE AND KETONE BODIES IN DIABETIC KETOACIDOSIS

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Abstract. Arterial blood lactate, pyruvate, plasma β-hydroxybutyrate and acetone have been followed in 12 patients with diabetic ketoacidosis during the initial 24-hour treatment period. In all cases the acidosis was fully explained by the accumulation of ketone bodies, and blood lactate and pyruvate never exceeded normal values. A slight rise was, however, observed in blood lactate in some patients during the treatment. Some possible explanations of this phenomenon are discussed. β-hydroxybutyrate was cleared from the plasma much faster than acetone, and the ratio between the ketone bodies was constantly changing during treatment of the acidosis.

For several years it has been known that in diabetic subjects acidosis can be caused by two sorts of derangement in the intermediary metabolism of carbohydrate and fat, i.e. ketoacidosis and lactic acidosis. The pathogenesis of the latter condition is largely unknown; it may appear in non-diabetics as well and is most often seen in patients severely ill from an infectious disease or in shock (8). Since both these conditions may be present in diabetics with ketoacidosis it is natural to assume that in some patients lactic acid might contribute to the acidosis. When this study was initiated, the question had not been thoroughly investigated, but recently Watkins et al. (10) have presented evidence that in patients with diabetic ketoacidosis blood lactic acid is normal. Our study confirms and extends these observations.

MATERIAL AND METHODS

The patients investigated were 12 diabetics, 6 males and 6 females, aged 18-76, admitted to the Medical Department with varying degrees of ketoacidosis. None of the patients had kidney disease.

The clinical and biochemical state of the patients at admission is presented in Table I together with the amounts of insulin, intravenous fluid and lactate administered in each case during the first 24 hours of treatment.

Treatment was started as soon as possible after admission. In all patients except one (case 12) insulin was administered with 40 I.U. intravenously every hour for the first hours and later subcutaneously. Intravenous fluid was given as isotonic NaCl and "hypotonic hydration fluid" (containing 100 mEq Na^+, 70 mEq Cl^- and 30 mEq lactate per litre).

Before treatment and after 2, 6, 14 and 24 hours arterial blood samples were drawn for determination of blood lactate, pyruvate, pH, pCO_2 and glucose and plasma β-hydroxybutyrate, acetone, standard bicarbonate, Cl^-, Na^+ and K^+. Urine samples were investigated for glucose (Clinistix®), acetone (Acetest®) and acetoacetate (the Gerhardt reaction).

Blood lactate and pyruvate were determined by enzymatic methods utilizing reagents from Boehringer Corporation Ltd. (3, 6). Plasma β-hydroxybutyrate was determined by a modification (1) of the enzymatic method of Gibbard and Watkins (4). Plasma acetone was determined by gas chromatography. With the procedure employed the acetone values give the sum of the acetoacetate and acetone in the sample, since acetoacetate decarboxylates spontaneously in the hot injector port of the gas chromatograph (2).

RESULTS

Before treatment of the acidosis all subjects studied showed a considerable elevation of plasma β-hydroxybutyrate and acetone, while blood lactate and pyruvate was found not to be increased. Before treatment plasma β-hydroxybutyrate was higher than plasma acetone in all cases except one (case 10). The difference between all measured cations and anions did not exceed what is generally found as cation-anion difference in the non-acidotic state (Table I).

In the first hours of treatment a sharp fall in plasma β-hydroxybutyrate was observed (Fig. 2), while the decrease in plasma acetone was slower

Table I *Clinical and biochemical state at admission and treatment in the first 24 hours of 12 patients with diabetic acidosis*

			Initial values in arterial blood							
Pat. no.	Age	Sex	Glucose (mg/100 ml)	pH	HCO_3^- (mEq/l)	Cl^- (mEq/l)	β-hydroxy-butyrate (mmol/l)	Acetone (mmol/l)	Lactate (mmol/l)	Pyruvate (mmol/l)
1	45	♂	790	6.98	6.0	103	11.6	8.1	0.98	0.103
2	21	♂	620	6.98	7.5		9.5	7.4	0.33	0.150
3	25	♀	516	7.09	8.2	95	12.6	10.5	1.30	0.019
4	41	♀	540	7.10	11.5	91	10.9	3.6	0.51	0.008
5	18	♀	277	7.11	8.0	107	9.2	8.3	0.31	
6	21	♂	845	7.16	8.2	92	18.8	11.0	0.83	
7	76	♂	1 200	7.21	13.2	100	12.4	7.4	1.04	0.24
8	67	♀	520	7.27	12.4	102	9.5	7.3		
9	48	♂	1 000		13.1	75				
10	69	♀	332	7.30	15.1	109	3.5	4.2	0.36	0.003
11	70	♀	880	7.31	14.4	91	10.5	8.2	0.51	0.001
12	59	♀	430	7.42	20.7	90	6.6	2.9	0.74	

and sometimes even preceded by a transient rise (Fig. 1). Blood lactate increased in five of eight patients in whom it was followed during all 24 hours (Table II), but normal values were never exceeded. Blood pyruvate values likewise were low and showed no characteristic pattern of change during the observation period.

In each case, as long as the correction of the acidosis progressed, a linear relation was found between arterial plasma standard bicarbonate and the sum of plasma acetone and β-hydroxybutyrate (Fig. 3).

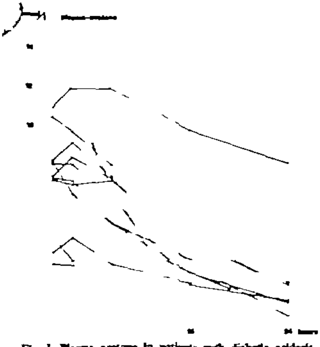


Fig. 1. Plasma acetone in patients with diabetic acidosis during the first 24 hours of treatment.

DISCUSSION

The question whether lactic acid may contribute to the acidosis in patients with diabetic keto-acidosis is sporadically brought up in the older literature (5). However only a single publication concentrating on this problem has appeared previous to our study (10). In accordance with this investigation we found, in our series of 12 diabetics with ketoacidosis at admission, blood lactate and pyruvate concentrations which did not exceed normal values. Thus lactic acid and pyruvic acid seem to be of no importance for the acidosis in diabetic ketoacidosis.

The increase in blood lactate during treatment of diabetic ketoacidosis has been found also by Watkins et al. (10). In our case it might be caused by the parenteral administration of fluids containing lactate, although no correlation was found between the amount of lactate administered and the variations in blood lactate (Tables I and II). A rising blood lactate has been demonstrated during the elimination of intravenously administered glucose in normal subjects (7). The increase in blood lactate seen during treatment in some patients in the present series might thus be

I	K (mEq/l)	Na (mEq/l)	Total cation (mEq/l)	Cation-anion difference (mEq/l)	Urine analyses[a]	Clinical state at admission	Duration of diabetes (y.)	Treatment within the initial 24-hour period: Insulin (I.U.)	Parent. fluid (l)	Lactate (mmol)
7	1 1	130	131 1	1	430	comatose	0	420	10.5	210
	3.7	135			431	drowsy	2	280	6.0	190
6	2.8	137	139.8	12	431	wake	13	440	8.9	132
5	4.9	126	130.9	13	431	drowsy	13	200	4.0	120
	2.2	134			431	drowsy	6	440	6.7	120
	4.8	124			431	comatose	0	560	8.0	90
3	6.0	143	149.0	16	410	drowsy	0	380	9.0	240
	4.7	128			430	awake	0	300	5.0	190
	2.3	125			430	drowsy	0			
	3.9	134	137.9	6	430	wake	0	40	1.0	30
.6	4.3	*128*	*132.3*	7	430	*drowsy*	40	200	3.0	90
.0	2.7	131	133 7	13	430	awake	20	40	1.0	30

he first column indicates the grade of glucosuria, i e. 4 = + + + + 3 = ++ The next columns indicate the Legal and ardt reactions.

attributed to the intense glucose elimination induced by the administration of insulin.

One might speculate whether unrecognized organic acids produced in the intermediary metabolism of carbohydrate and fat might be a contributory factor in the development of acidosis in diabetic ketoacidosis. Our study showed that before treatment the cation-anion difference (calculated as the sum of all measured cations minus the sum of all measured anions) was of the same order of magnitude as the anion gap in the non acidotic subject (9). This finding indicates that no large amounts of anions not measured by our techniques were present in the blood of the patients. Furthermore in every patient investigated we found during treatment of the acidosis a linear relationship between plasma standard bicarbonate and the sum of plasma ketone bodies. These two observations seem to indicate that neither before nor during treatment of diabetic ketoacidosis are other acids than ketone bodies present in amounts affecting acid-base balance.

The plasma concentration curves of β-hydroxybutyrate have a somewhat exponential shape as compared with the acetone curves, which tend to be more linear sometimes with a transient initial rise. This is in accordance with the pathways of ketone catabolism, in which acetoacetate is formed from β-hydroxybutyrate. It would seem that during

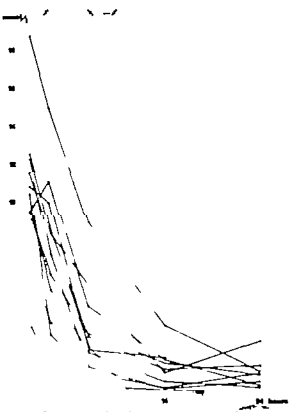


Fig. 2 Plasma β-hydroxybutyrate in patients with acidosis during the first 24 hours of

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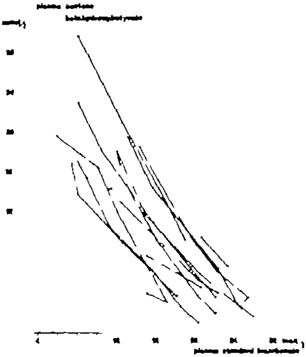


Fig. 3. The sum of plasma acetone and β-hydroxybutyrate values in relation to plasma standard bicarbonate during treatment of patients with diabetic acidosis.

initial insulin treatment of diabetic ketoacidosis β-hydroxybutyrate conversion to acetoacetate is a much faster process than is the disappearance of acetoacetate and acetone.

It is evident from the different shape of the curves in Figs. 1 and 2 that the ratio β-hydroxybutyrate/acetone is constantly changing during treatment of the acidosis. Williamson et al. (11) and Gibbard and Watkins (4) found the ratio β-hydroxybutyrate/acetoacetate by enzymatic determinations to be rather constant in plasma in non-acidotic and slightly ketotic subjects. Although in our study we have estimated acetoacetate and acetone together it would seem that Watkins et al. (10) are incorrect when they are assuming that the constancy of the ketone ratio can be extended also to more severe acidosis.

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Table II. *Blood lactate (mmol/l) in patients with diabetic acidosis during the first 24 hours of treatment*

Pat. no.	0 h	2 h	6 h	14 h	24 h
1	0.98	0.79	0.77	—	0.65
2	0.33	0.53	0.72	0.76	1.19
3	1.30	1.04	0.69	0.55	0.78
4	0.51	0.75	0.45	0.20	—
5	0.31	0.20	0.44	0.65	0.40
6	0.83	1.30	—	0.26	0.32
7	1.04	0.75	0.82	0.97	0.46
9	—	4 h 0.54	6 h = 1.44	10 h = 0.74	18 h = 0.37
10	0.36	0.43	—	—	—
11	0.51	0.86	0.94	0.82	0.39
12	0.74	0.70	—	—	—

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TREATMENT OF SEVERE HYPERTENSION WITH CATAPRES® (St 155)

A Comparative Study of the Effect of Catapres and Methyldopa in a Series of Hypertensive Outpatients (WHO Stage III)

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Abstract. A comparative study of the effect of Catapres® (clonidine BAN) and methyldopa has been carried out in 23 patients suffering from severe hypertension (WHO Stage III). Each patient was observed for a period of 5 months, during the course of which the following parameters were investigated: blood pressure in supine and erect position, renal function (^{51}Cr-EDTA and ^{131}I hippuran clearance), liver function, serum electrolytes, ECG, and ophthalmoscopy. Furthermore the side effects have been recorded. No significant differences in the effect of the two drugs were noted concerning blood pressure, renal function and the other parameters mentioned. Methyldopa presented a higher frequency of severe side effects than did Catapres.

Recently a new antihypertensive drug, Catapres® (clonidine BAN, St 155) has been introduced by Boehringer Ingelheim. Catapres is a derivative of imidazoline and it has been found to possess potent hypotensive properties when given in microgramme dosages. Normally the reduction in blood pressure is associated with a reduction in cardiac output and a decrease in peripheral resistance. The pharmacodynamical site of action of Catapres is believed to be a superior centre in the central nervous system. The main side effects of St 155 are sedation and dryness of the mouth. The toxicity of Catapres is extremely low, the oral LD_{50} for mice and rats being about 50 000 times the normal human daily dose. The effect of St 155 is antagonized by tolazoline.

During recent years, Scandinavian (2, 6, 8–10) and other authors (7, 9, 14) have described the pharmacological and clinical effects of St 155 and for further information we also refer to the proceedings from two symposia recently held on Catapres (3, 5).

MATERIAL AND METHODS

The study comprises 23 hypertensive patients (13 females and 10 males) admitted to the Department of Medicine during 1968. Their ages ranged from 20 to 70 years, average age 47 years. All patients who were able to come to the clinic, and who suffered from so severe hypertension that they could not be treated successfully with diuretics alone, were included. On the whole, the material must be classified as WHO Stage III, which covers patients having high blood pressure with evidence of organ damage attributable to the hypertensive disease (13).

On admission, any antihypertensive therapy was discontinued and the following investigations were made: measurement of systolic and diastolic blood pressure, X-ray of the heart, ECG, ophthalmoscopy, determination of alkaline phosphatase, GOT, sodium, potassium, standard bicarbonate, and creatinine in serum. Furthermore renal function was determined by measurements of the glomerular filtration rate and the renal plasma flow. Following these examinations, treatment was instituted. All patients were given Hygroton® (chlorthalidone), 50 mg every second day. This basal therapy was maintained throughout the experiment. Furthermore, 14 patients who had received methyldopa before admission, were given Catapres. The remaining 9 patients who had previously been treated with drugs other than methyldopa, or who had received no antihypertensive therapy were given methyldopa. The patients remained in the ward until a distinct reduction in blood pressure was obtained.

After discharge the patients continued the treatment instituted. Unless there was a lack of response to the drug or side effects appeared which necessitated termination at an earlier time, the treatment was continued over the next 2 months. After this period, the alternative drug (Catapres or methyldopa) was given, and this also was continued for 2 months. An exception was made in 3 patients because, just before the start of the experiment, they appeared refractory to methyldopa and consequently were not treated with this drug. Hence these patients are not included in Tables I and IV. At the end

Table I *Initial and control values of 20 patients*

			Hospital period								Outpatient period							
			Untreated								Catapres + Hygroton				Methyldopa + Hygroton			
							Renal function				MBP		Renal function		MBP		Renal function	
Case no.	Age	Sex	Fundus grade	Serum creatinine (mg/100 ml)	Heart enlarge-ment	MBP supine (mmHg)	GFR (ml/min)	RBF (ml/min)	Treated MBP supine (mmHg)		Supine (mmHg)	Erect (mmHg)	GFR (ml/min)	RPF (ml/min)	Supine (mmHg)	Erect (mmHg)	GFR (ml/min)	RPF (ml/min)
1	47	♂	III	4.4	+	150	23.1	8.0	112 (C)		150	138	20.6	34.5	149	138	17.3	29.2
2	47	♀	II	1.2	–	139	82.4	363.7	112 (C)		123	118	71.5	209.3	137	142	83.3	253.4
3	52	♂	II	1.2	–	140	108.1	486.7	116 (C)		127	119	88.6	483.6	147	124	96.2	454.1
4	47		II	0.8	–	129	—	—	110 (M)		114	92	—	—	112	101	—	—
5	27	♂	0	0.8	–	144	177.2	539.7	115 (M)		121	113	112.7	479.0	121	106	110.6	524.3
6	38	♂	0	1.0	–	122	117.9	562.6	110 (M)		114	103	108.1	425.0	104	106	107.2	396.1
7	40		I	1.5	–	137	44.0	165.1	108 (M)		116	89	39.6	132.8	113	94	35.6	147.5
8	56	♀	II	1.9	–	144	33.4	128.3	118 (C)		140	129	32.0	121.9	130	112	32.4	126.7
9	44		II	1.6	+	165	—	—	114 (M)		148	99	—	—	155	109	—	—
10	43	♂	I	1.0	–	148	46.9	98.1	114 (C)		120	110	44.4	129.1	140	125	42.3	130.7
11	34	♀	0	1.0	–	137	113.1	544.5	103 (M)		118	107	—	—	113	103	108.8	497.4
12	37	♂	II	0.6		158	137.9	556.2	94 (C)		144	125	121.3	501.8	134	120	112.0	501.8
13	66	♂	II	2.1		156	67.7	235.6	158 (C)		184	143	—	—	173	149	56.7	—
14	70	♂	II	1.9	–	166	58.6	174.8	141 (M)		177	115	44.2	163.5	165	128	—	—
15	60	♂	II	5.5	–	148	34.4	74.9	138 (M)		155	112	17.7	47.3	168	134	—	—
16	45	♀	0	1.4	–	148	51.2	229.1	118 (C)		127	118	46.7	239.5	—	—	—	—
17	57	♂	II	1.0	–	142	—	—	97 (C)		122	93	—	—	—	—	—	—
18	45	♀	I	0.9	–	150	—	—	118 (C)		139	125	—	—	—	—	—	—
19	47	♂	III	1.6	–	148	73.5	241.0	135 (C)		145	131	48.1	280.6	—	—	—	—
20	47	♂	II	1.2		153	125.8	424.2	1 4 (M)		156	121	105.3	373.6	—	—	—	—

MBP Mean blood pressure. GFR = Glomerular filtration rate. RPF Renal plasma flow
(C) Catapres treatment. (M) Methyldopa treatment.

Table II. *Comparison between MBP during hospital stay and in outpatient period in 12 patients*

(*a*) *MBP mean and S.D*

Treatment	Hospital period —	Catapres	M-dopa	Outpatient period Catapres		M-dopa	
No. of pats.	12	6	6	12		12	
MBP measured	Supine	Supine		Supine	Erect	Supine	Erect
Mean (mmHg)	143	111		128	112	130	115
S.D. (mmHg)	12	7		14	15	17	15

(*b*) *T Tests on supine MBP*

Hospital period	Outpatient period Catapres	M-dopa
Untreated	$p < 0.01$	$p < 0.05$
Treated	$p < 0.001$	$p < 0.005$

of each of the 2-month periods, examinations were made, including all parameters mentioned above. Furthermore, during the outpatient period, the patients were examined once week, on which occasion the blood pressure was recorded in the patients after 5 min in supine position and after standing for 1 and 3 min, and the patients were questioned systematically with respect to side effects. At these control examinations, which were all carried out by the same examiner (F B.-C.), the dosage of antihypertensives was changed as required.

Only 12 of the 23 patients were able to complete 2-month period on *both* antihypertensive drugs. In six cases treatment had to be discontinued because of lack of effect, and in five cases methyldopa had to be withdrawn because of serious side effects.

RESULTS

Blood pressure measurements

For the evaluation of the blood pressure level, the mean blood pressure (MBP) was employed, which signifies the sum of the diastolic blood pressure and one third of the amplitude. For the outpatient period, the blood pressure readings are given as the average of the last five measurements during the period concerned. The average values for the blood pressure readings with the patient in the standing position were consistently calculated on the basis of the lowest value of MBP after standing for 1 min or 3 min. The MBP values for the different test periods are shown in Table I.

Table II presents the effect of the two drugs on the MBP in the 12 patients who completed both outpatient periods. In measurements with the patient in the supine position, an average value during Catapres treatment of 128 mmHg was found and, during methyldopa treatment, of 130 mmHg. The corresponding measurements in the standing position were 112 for Catapres and 114 mmHg for methyldopa, and these MBP values present no statistically significant difference. Furthermore, it appears from T ble II *a* that, during the hospital period, the 12 patients presenting an average MBP in the supine position of 143 mmHg when untreated had this value reduced to 111 mmHg during treatment. Both these values show a statistically significant difference from the MBP values in the supine position during the outpatient period (128 and 130 mmHg), as will be seen from Table II *b*

Table III shows the distribution of the 23 patients classified according to the effect on the MBP "Satisfactory response was defined by the following criteria.

Patients under 40 years: supine MBP ≤ 115 mmHg.

Patients between 40 and 50 years: supine MBP ≤ 120 mmHg.

Patients over 50 years: supine MBP ≤ 130 mmHg.

At discharge from hospital 15 of the 23 patients had a satisfactory blood pressure (Table

Table III. *Effect of antihypertensive treatment in 23 patients*

	Hospital period		Outpatient period	
	Catapres	M-dopa	Catapres	M-dopa
Satisfactory response of MBP	9	6	9	6
Less response of MBP	4	3	6	6
No effect	1	—	8	6
Excluded due to severe side effect	—	—	—	5
Total	14	9	23	23

III). Immediately after discharge all the patients experienced a rise in blood pressure.

During the outpatient treatment with Catapres 9 patients presented satisfactory MBP values, 6 had a less pronounced, but not satisfactory fall in blood pressure, and in 8 the MBP was equal to, or higher than, the MBP observed during the non-treatment period of hospitalization. When evaluating the effect of methyldopa, it should be borne in mind that, out of 23 patients, treatment had to be discontinued in 5 before the end of the period because of side effects. Of the remaining 18 patients, 6 responded satisfactorily to treatment, whereas another 6 presented a less pronounced, but not satisfactory fall in blood pressure, and 6 were refractory to treatment.

Renal function

The results of the initial serum creatinine values and the renal function appear in Table I. During treatment with either one of the hypotensive drugs an increase in serum creatinine of an average of 0.1 mg/100 ml was observed, as compared with the values during the period of no treatment.

In most of the patients, the nephropathy must be regarded as secondary to the hypertensive disease. However 2 patients had chronic pyelonephritis and 1 had glomerulonephritis.

The glomerular filtration rate was measured by means of the ^{51}Cr EDTA method (1, 4) and the renal plasma flow by means of the ^{125}I hippuran or para-amino-hippuric-acid clearance (11, 12) in a total of 16 patients, in 14 patients before and after Catapres treatment, and in 11 before and after treatment with methyldopa. The initial glomerular filtration rate varied from 23 to 138 ml/min, and the plasma flow from 28 to 563 ml/min.

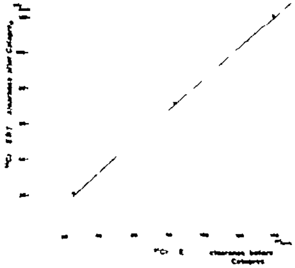


Fig. 1. ^{51}Cr-EDTA clearance before and after Catapres.

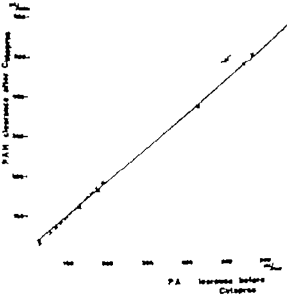


Fig. PAH-clearance before and after Catapres.

Figs. 1 and 2 present the effect of Catapres + Hygroton on the clearance values. A rectilinear relationship between the values before and after Catapres treatment is seen, with a coefficient of correlation of ≥ 0.98. A statistical examination was made of whether the slope of the correlation curve differed from 1.0. It appeared that both the glomerular filtration rate and the plasma flow were reduced during Catapres treatment ($0.01 < p < 0.025$ and $0.005 < p < 0.01$ respectively).

A similar analysis of the figures from the 11 patients in whom the renal function tests were made before and after methyldopa treatment showed that methyldopa + Hygroton reduced the glomerular filtration rate and the renal blood flow correspondingly ($0.01 < p < 0.025$ and $0.01 < p < 0.025$ respectively). The two antihypertensive drugs reduced the renal function by 10–15% and this reduction did not seem to depend on the previous level of function.

Electrocardiography

In 4 patients the ECGs were normal, while left axis deviation was found in 4 patients. Seven patients presented signs of myocardial ischaemia, and in 8 patients left ventricular strain was observed.

During treatment with Catapres the signs of ischaemia diminished in 3 patients, whereas in 2, the initial signs of ischaemia presented aggravation.

During treatment with methyldopa, 4 patients had signs of diminished ischaemia, whereas none had increased ischaemia.

Ophthalmoscopy

For initial values see Table I. No essential alterations in the retinal fundus occurred in any of the patients during the study with the exception of one in whom the changes in the fundus decreased from grade II to I during treatment with both drugs.

Dosage

In the 14 patients who commenced treatment with Catapres during the stay in hospital, the average dose was 0.340 mg/24 h, increasing to 1.050 mg/24 h at discharge. During the outpatient period the average dose was somewhat higher and a maximum 24-hour dosage of 1.950 mg Catapres was reached.

In the 9 patients who began with methyldopa during the stay in hospital the average initial dose was 600 mg/24 h, increasing to approximately 1 500 mg/24 h at discharge. During the outpatient period, daily doses as high as 4 000 mg methyldopa were given. However doses of this magnitude were given over brief periods only since no definite effect was observed in cases where the daily dose exceeded 2 500 mg.

When changing from one drug to the other 0.150 mg Catapres was regarded, on principle, as equivalent to 250 mg methyldopa.

Side effects

Table IV presents the frequency of side effects in the 20 patients treated with both drugs.

As regards Catapres, the treatment with this drug could be completed without having to discontinue medication in any of the patients because of side effects. A particular effect of Catapres was observed in several patients in the form of some facial pallor.

As regards methyldopa, the treatment had to be discontinued in 5 patients because of side effects. In 2 of these cases the cause was pronounced nausea, vomiting and diarrhoea to such a degree that the patients after 1 or 2 days had to discontinue the treatment. A third patient, who presented the same symptoms, had to abandon treatment after 2 weeks. In a fourth case, treatment with methyldopa had to be stopped after 1 week because of general discomfort from fatigue, sedation, dryness of the mouth, depression and headache. Finally the fifth patient received methyldopa for 3 weeks only after which period, in spite of a fairly acceptable blood pressure level, another drug had to be given because the patient complained of an unendurable neuralgiform headache. However this patient had suffered from very severe headaches for several years, resulting in several admissions to neurological departments. After having changed to Catapres the patient has only a very slight headache. Furthermore, when Catapres treatment was discontinued, several patients complained of headache, not necessarily accompanied by a rise in blood pressure. If Catapres was suddenly withdrawn, nausea and general discomfort were observed.

During treatment with either of the antihyper

Table IV *Side effects in 20 outpatients*

Treatment	Catapres			Methyldopa		
Sideeffects	None	Slight	Severe	None	Slight	Severe
Dizziness	11	9	—	14	5	1
Nausea/vomiting	20	—	—	17	—	3
Sedation	4	14	2	9	6	5
Sleep disturbances	10	9	1	15	3	2
Dryness of mouth	6	10	4	12	4	4
Obstipation	17	3	—	20	—	—
Diarrhoea	20	—	—	17	2	1
Mental depression	19	1	—	16	2	2

tensive drugs the clinical and laboratory studies did not indicate any toxic influence on the retina, the liver or the haematopoietic system. Electrolyte studies showed signs of hypokalaemia which must be ascribed to the basal therapy with chlorthalidone.

DISCUSSION

In the present group of hypertensive patients a less pronounced blood pressure reducing effect was found than that normally seen in similar studies of the two drugs used. Presumably this can be explained by the fact that our material comprised patients suffering from very severe hypertension, most often with one or more serious hypertensive stigmata. It is a well known fact that the presence not only of nephropathy but also of retinopathy increased cardiac index and abnormal ECG makes it less easy to obtain a satisfactory fall in blood pressure by drug treatment. This study also confirms the fact that a stay in hospital has a favourable effect on the blood pressure in contrast to the much higher blood pressure level recorded in patients carrying out their normal activities. Since our patients were examined late in the afternoon, most often on their way home from work, higher blood pressure readings than expected were registered, and furthermore the values often varied considerably from one control to the other However this type of blood pressure recording may provide the most realistic picture of the effects of any treatment.

In the present study it was found that both the glomerular filtration and the renal plasma flow decreased during the antihypertensive therapy The decrease was within the range of 10 to 15% and in this respect no difference in the effect of the two drugs was recorded. However it should be pointed out that about half of the patients suffered from renal insufficiency presumably of a progressive nature.

Regarding side efects, Catapres was tolerated well by all patients, whereas in many patients methyldopa gave rise to so serious side effects that this therapy had to be discontinued.

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THE INFLUENCE OF DIETARY FATS ON PLATELETS IN MAN

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Abstract. Two groups, each of 10 subjects, have been given isocaloric diets for 21 days. The diets were identical with the exception of their fat content. One group was given 40% of the calories as soybean oil, and the other the same amount as MCT oil. Platelet and serum lipids and platelet function tests were evaluated before and after the dietary period. The platelet lipid analysis included estimation of total cholesterol and phospholipids, fractionation of phospholipids by thin layer chromatography and gas liquid chromatographic estimation of the fatty acid and aldehyde composition of the various phospholipids. Platelet function tests included platelet factor 3 (PF 3) activity and availability, platelet aggregation induced by collagen, ADP and thrombin and platelet electrophoresis. In subjects given soybean oil diet significant reduction in serum total cholesterol and phospholipids occurred. No changes were observed in the platelet cholesterol and phospholipid levels, whereas significant increase of linoleic acid in choline, ethanolamine and serine phosphoglycerides took place. The relative increase of linoleic acid was most marked in the choline phosphoglycerides. A moderate decrease in palmitic and oleic acids was seen in most phospholipid fractions. PF 3 activity in platelet-rich plasma showed consistent decrease in these subjects, whereas the other platelet function tests showed insignificant changes. In subjects given MCT oil diet no significant changes were observed in serum or platelet lipids, including the phospholipid fatty acids. Increased PF 3 activity in platelet-rich plasma exposed to ADP was the only functional disturbance observed in this group.

When platelets aggregate, the platelet phospholipids are made available for plasma coagulation (27). These phospholipids, which are mainly located in platelet membranes and granules, are thus involved in an important step in thrombus formation (22, 28). Recent studies indicate that platelets contain enzymes for a complete fatty acid synthesizing system and for a de novo phospholipid synthesis (18, 21). It has also been shown that platelets incubated with ^{14}C-labelled fatty acids in vitro are able to incorporate these fatty acids in neutral lipids and phospholipids (4). The distribution pattern of the labelled fatty acids was in most cases similar to those of the fatty acids found in natural platelet phospholipids. These studies clearly indicate that platelets are different from other mature cells in the blood in respect of their ability to synthesize phospholipids (20, 32).

Animal studies indicate that dietary fats influence the fatty acid composition of phospholipids in liver microsomes and mitochondria (19, 40), in red cells (8), brain tissue (39), platelets (26), and in experimentally induced atherosclerotic lesions (37). Experimental studies further indicate a relation between platelet phospholipid fatty acid composition and platelet function. Rats fed highly saturated fat diets showed a high incidence of ADP-induced platelet thrombi compared to animals fed isocaloric diets rich in polyunsaturated fat (26).

The present study deals with the influence of dietary fats on platelet lipids and platelet function in man.

MATERIAL AND METHODS

Twenty subjects, 18 males and 2 females, aged 23 to 55 years, participated in the dietary study. The subjects included healthy medical students and patients with chronic atherosclerotic diseases. The subjects were randomly divided into two groups, each of 10 persons, with an equal number of healthy subjects and patients in each. During a period of 21 days they received all their meals at the dietary kitchen in the hospital. All subjects were motivated for the study and ingestion of additional food or drugs during the dietary period was excluded. During the study all tests were run on paired subjects, one on each diet. Each subject represented his own control.

Table I. *Composition of experimental diets*

	(g)	Protein (g)	Fat (g)	Carbo-hydrate (g)	Kcal.
Breakfast and evening meal					
Bread	230	22	3	120	598
Lean meat	60	12	1.2		60
Skimmed milk	300	10.5	0.3	15	105
Dried skimmed milk	10	3.7	0.1	5.3	37
Jelly	60	0.4		24	97
Sugar	5			5	20
Puffed wheat	25	1.4	0.3	22	97
Dinner					
Lean meat or	205	35	3.3		205
Lean fish	290				
Potatoes	150	2.7	0.1	25	115
Vegetables	150	1.5	0.2	10	60
Wheat flour	10	1	0.1	7	34
Fruit	150	0.6	0.1	30	120
Additional fat					
Soybean oil or MCT oil	112		112		1 000
Total		91.8	121.5	263.3	2 548

Diet

Two diets were used in this study. They were isocaloric and contained 40% fat. The only difference between the diets was that the fat source in one was soybean oil and in the other oleum vegetabile tenue (MCT oil) (Dansk Soyakage Fabrik, Copenhagen). The compositions of the diets are given in Table I. The fatty acid compositions of the main dietary fats were as follows: soybean oil 16:0 (10%), 18:0 (4%), 18:1 (17%), 18:2 (60%), 18:3 (9%), and MCT oil 8:0 (46%), 10:0 (48%), 12:0 (6%). The soybean oil had an iodine value of 133 and the MCT oil an iodine value of 4. The oils were incorporated in the diet by preparation of "oil-butter" and sauces.

Blood collection

Venous blood was collected after the subjects had been fasting for 14 hours, at the start and at the end of the dietary period. Serum was prepared from 10 ml of blood and used for estimation of the main lipid fractions and lipoproteins. Forty-five ml of blood was collected in 5 ml of 0.106 M citrate solution and used for platelet function tests. Thirty-six ml of blood was collected in 4 ml of 0.077 M EDTA solution, pH 6.4 and used for preparation of platelet concentrate for lipid analyses.

Platelet function tests

Platelet factor-3 activity was estimated in platelet-rich and platelet-poor plasma with standardized platelet counts with and without exposure to kaolin, ADP and freezing and thawing three times. The method of Hardisty and Hutton (13) with slight modifications (27) was followed. *Electrophoretic mobility* of platelets and erythrocytes and plasma viscosity were estimated according to the methods described by Grøttum (10) and Grøttum and Solum (11). *Platelet aggregation* was studied by the turbidimetric method of Born (2). PRP was exposed to 1) collagen solution (collagen from tendon, Sigma Chem. Comp. St. Louis, Miss.) prepared by homogenization in 0.5% acetic acid and dilution with 0.1% acetic acid, giving a final collagen solution of 0.125 mg/ml, pH 3.1 and a final concentration of collagen in PRP of 4.2 μg/ml, 2) ADP (Sigma) in a final concentration of 0.3 μg/ml PRP and 3) thrombin (Topostasin® SAF Hoffmann La Roche, Basel, Switzerland) in a final concentration of 0.3 N.I.H. U/ml PRP. As shown in Fig. 1 the slope of the curve was estimated, and the distance in mm was used as measure for aggregation. Preliminary studies have shown a linear relationship between the platelet count and the slope of the curve with platelet counts in PRP between 100 $\cdot 10^9$ and 500 $\cdot 10^9$. The estimated values, therefore, were corrected to a platelet number of 300 $\cdot 10^9$. *Platelets counts* were done in a Linson Celloscope (Linson Instrument AB, Stockholm).

Serum lipids

Total cholesterol (3), triglycerides (16), phospholipids (9), unesterified fatty acids (7) and lipoproteins (25) were measured by methods described earlier.

Platelet phospholipids

The method has recently been described in detail (27). By differential centrifugation a platelet concentrate was prepared which was washed twice. The platelets were counted before the extraction of lipids was performed. α-tocopherol was added to all samples to avoid autooxidation. The phospholipid distribution was determined in triplicate by thin layer chromatography (TLC) as

nitrogen atmosphere at 4°C. The chromatogram was developed in chloroform–methanol–acetic acid–water (25:15 4 2). Various staining procedures, standard phospholipids and two-dimensional TLC were used for identification. The amount of lipid phosphorus was determined by Marinetti' modification (23) of Bartlett's method (1).

Platelet total cholesterol

One hundred μl of washed platelet concentrate was frozen and thawed three times and extracted in 2.5 ml of 2 1 (v/v) chloroform–methanol. After initial shaking, the tubes were allowed to stand for 120 min with oc casional shaking at room temperature. The solution was then evaporated to dryness under nitrogen in 18 150 mm test tubes and 4.7 ml of 95% ethanol and 0.3 ml of 33% aqueous KOH were added. The tubes were shaken vigorously and placed in water bath at 55°C for 15 min. The tubes were cooled briefly and 5 ml of distilled ater and 10 ml of hexane ere added. The solutions were mixed for 30 sec on whirlimixer and allowed to stand for separation. Five ml of the upper hexane phase were transferred to 19 65 mm screw cap vial and evaporated to dryness using dry nitrogen. Fifty µl f internal standard (50 mg 5α-cholestane (Mann Res. Labs. Orangeburg, N.J.) in 100 ml chloroform) were added to the dry residue and, following mixing to get homogenous solution, 3 μl are injected into the gas chromatograph. A cholesterol standard was run for every tenth sample An F & M Scientific Instrument Model 402 equipped with 2 ft 5 mm d. glass column and packed with 3.8% silicone Gum Rubber Methyl—GESE —30 on GAS-Chrom Q, 80/100 mesh, was used. Argon was used as carrier gas and the column temperature was maintained at 225°C. The peak heights of cholestane and cholesterol were measured and used for calculation of cholesterol

Platelet phospholipid fatty acids and aldehydes

Some minor modifications have been added to the original methods (27). The phospholipid spots from the TLC procedure were used for preparation of methyl esters and dimethyl acetals from boron trifluoride methanol. Gas liquid chromatography (GLC) was performed on an F M Scientific Instrument (Hewlett-Packard) Model 402 equipped with paired glass columns, 6 ft 5 mm i.d. and packed with 10% EGSS-X on 100–120 mesh Gas-Chrom (Applied Sciences Lab. Inc., State College, Pa., USA). Programmed runs from 165 to 195°C were used. Peak areas were calculated with planimeter and NIH fatty acid methyl ester standard mixtures were used to control the quantitation and as references.

Platelet protein analysis

The protein estimation was carried out on aliquots of washed platelet concentrate as described earlier (27).

Statistics

The *t*-test applied to paired comparison was used for evaluation of the results

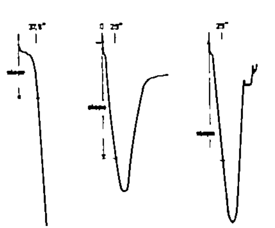


Fig. 1 Platelet aggregation induced by collagen, ADP and thrombin. Tracing f aggregation curves with super position of vertical line through the zero point. The slope as defined as the mm of fall over 37.5 sec (collagen) and 25 sec (ADP thrombin) period from zero point. Concentrations of test agents given under Methods.

RESULTS

All subjects were in excellent condition and no significant weight changes occurred during the dietary periods.

Serum lipids

All persons had a normal lipoprotein pattern. As shown in Table II, the subjects given a soybean oil diet had an average reduction of total cholesterol and phospholipids of 22 and 21% respec tively whereas no significant changes were observed in subjects given MCT oil diet. All subjects given soybean oil showed this reduction even if their initial cholesterol levels in many instances were below or close to 200 mg/100 ml.

Platelet factor-3 activity

A significant, though moderate, decrease in platelet factor 3 activity was found in PRP and PPP from subjects given soybean oil (Table III), whereas subjects given MCT showed a non-significant increase ($0.1 > p > 0.05$) of PF-3 activity in PRP The activity of PF-3 after exposure of PRP to kaolin and ADP and the "total" PF-3 activity measured after freezing and thawing three times showed the same tendency However the dif

Table II. *Serum lipids before and after a 21-day period on soybean oil and on MCT oil diet (n = 10)*

	Soybean oil		MCT oil	
Serum lipids	Before	After	Before	After
Total cholesterol (mg/100 ml)	238±44	185±30*	225±58	216±33
Triglycerides (mg/100 ml)	69±35	67±32	74±31	87±45
Phospholipids (mg/100 ml)	16±35	170±26	211±60	212±36
Unesterified fatty acids (μmol/l)	531±255	477±330	536±201	530±287

* $p < 0.01$

ferences were not significant, except for the ADP reaction in subjects on the MCT diet, and the total" activity in subjects given soybean oil.

Platelet electrophoretic mobility

The electrophoretic mobility of platelets and erythrocytes and the relative plasma viscosity were estimated in 5 subjects from each group. Even if the number of tested subjects is small, the results were most consistent and showed no changes during the dietary periods.

Platelet aggregation

The estimation of platelet aggregation after exposure of platelet rich plasma to collagen, ADP and thrombin showed a considerable variation in this group of 20 subjects before any dietary changes were instituted. This variation was particularly marked after exposure of PRP to collagen and thrombin. A tendency to increased aggregation in the MCT oil group was present, though no significant changes could be registered in either of the two groups.

Platelet phospholipids and cholesterol

As shown in Table IV the content of the various phospholipids and the percentage distribution of the platelet phospholipids were not significantly influenced by the dietary regimen.

An average cholesterol level of 107.3 ± 11 4 μg/10^9 platelets was present in the total group of 20 subjects before any diet was given. No significant changes of the platelet cholesterol was observed in either of the dietary groups, and the cholesterol/phospholipid ratio as well as the protein/phospholipid ratio remained unchanged.

Fatty acid composition of platelet phospholipids

The percentage distribution of the phospolipid fatty acids is given in Tables V–VIII. In subjects given soybean oil a significant increase of linoleic

Table III. *Platelet factor-3 activity in platelet-rich and platelet-poor plasma from subjects before and after the dietary period (n = 10)*

		Stypven time (sec)			
		Soybean oil		MCT oil	
No of platelets/mm³	Test reagent	Before	After	Before	After
300 000	Buffered saline	39.5±5 4	43.3±4.2	42 2±3.5	39 1±3.6
300 000	Kaolin (1 mg/ml)	27.2±3.0	27.3±2 4	28 1±3.6	27 8±1.9
300 000	ADP (2 10⁻⁴ M)	35.3±5.5	36.6±3.9	36.7±4.7	33.7±2.8
300 000	Frozen and thawed 3 times	16.5±1.5	17 8±1.3	17.0±1 5	16.8±1 3
15 000	Buffered saline	61.0±4 7	67.2±5.1	61.6±6.4	59.3±2.3

$p < 0.01$ $0.01 < p < 0.05$

able IV *Phospholipid composition of platelets from subjects on different diets (n = 10)*

	Soybean oil				MCT oil			
	Before		After		Before		After	
ompound	μg lipid-P / 10^9 platelets	%	μg/lipid-P / 10^9 platelets	%	μg lipid-P / 10^9 platelets	%	μg lipid-P / 10^9 platelets	%
E.	3.67±0.67	32.7	3.70±0.60	32.6	3.57±0.62	30.8	3.34±0.63	30.9
S.	0.81±0.22	7.1	0.73±0.36	6.7	0.93±0.46	8.1	0.93 ±0.32	8.8
I.	0.22±0.20	1.9	0.27±0.23	2.9	0.29±0.16	2.5	0.26±0.15	2.5
C.	5.11±0.97	45.4	5.09±1.02	44.5	4.94±0.69	42.9	4.64±0.61	43.2
ph.	1.47±0.50	12.9	1.59±0.36	13.9	1.80±0.35	15.6	1.58±0.34	14.6
otal rec. lipid-P*	11.3±2.3		11.4±1.7		11.5±1.4		10.7±1.5	

Represents 94.8±5.6% of total lipid-P

cid was observed in three of the main phospholipid fractions, with the most marked and consistent increase in the choline phosphoglycerides. n the same subjects a moderate decrease of palitic and oleic acid was seen. This pattern was resent in P.C., P.E. and P.S. The long chain, ghly unsaturated fatty acids (≥20·4), which articularly are present in P.E., showed no gnificant changes. The sphingomyelins showed a significant reduction of docosenoic acid (22.1) in the soybean oil group.

The subjects given MCT oil showed only very moderate changes in their phospholipid fatty acid pattern. A slight increase of palmitic acid in the P.E. fraction and a similar increase of stearic acid and tetracosenoic acid (24 1) in the sphingomyelins were registered. The medium chain fatty acids of the MCT oil diet were not reflected in the platelet phospholipids.

The ratio between saturated fatty acids plus aldehydes and unsaturated fatty acids showed no significant changes, though a tendency to a lowered ratio was seen in the phospholipids from subjects given soybean oil.

Table V *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of ethanolamine phosphoglycerides in latelets from subjects on different diets (n = 10)*

	Soybean oil		MCT oil	
Components	Before	After	Before	After
16 0 DMA	5.4±1.2	5.2±1.8	6.4±2.0	6 0±2.3
16:0	5 6±2.0	6.5±3.6	5.3±2.5	6 4±2.5
16.1	0 3±0.1	0 3±0.1	0 4±0.1	0 6±0 1
17.0	0.2±0.1	Trace	Trace	Trace
18.0 DMA	8.3±2.4	6 9±2.0	7 9±3.2	7.6±2.4
18:0, 18 1 DMA	28.4±7 1	29.3±6.2	26.7±5.3	29 6±7.6
18.1	12.0±2.9	11 1±2.2	12 5±4 6	11.8+3.3
18.2	3.2±1 2	4.5±1.3	2 9±0.9	2.6±1.6
20·0	0.9±0.1	1.0±0.1	0.7±0.1	1.0±0 8
20:1 18.3	1 4±0.9	1.9±0.6	1.6±0 7	1 8±1.4
20:3, 22.0	0.8±0.1	0 8±0.1	1.0±0.4	0.8±0.1
20.4, 22.1	21.8±8 2	23 8±4.7	24.2±9.2	24.1+8.9
20:5, 22.2	1.0±0.1	0.7±0.1	0.9±0.1	0 5±0.1
22.4	1.0±0.1	1 1±0.5	1.6±1.2	1.8±1.0
22.5	2.5±1.6	2 3±1 5	3.2±2.2	2.7±1 2
22·5	2.7±2 2	2.1±1 3	2 5±1.9	2.2±1 5
22.6	2 3±1 2	2 0+0.9	2.2±1.2	2.0±0 8
26.1	Trace			

p 0 02

Table VI. *Fatty acid distribution (g/100 g fatty acids and aldehydes) of serine phosphoglycerides in platelets from subjects on different diets (n = 10)*

	Soybean oil		MCT oil	
Components	Before	After	Before	After
16 0	10 1±7 7	8.9±4.5	7.2±4 2	5.9±4 5
16.1	1 1±0 2	0 8±0.1	1.2±0.3	0.4±0.1
17.0	Trace	Trace	Trace	Trace
18·0	48.3±4.5	50 6±11 4	51.3±4.2	53.7±6.9
18 1	23.2±7 1	19.3±11.8	21.0±5.1	20 1±7.8
18 2	1.8±0.6	4 0±2.1	1 1±0.6	1.6±0 4
20 0	1.5±0.6	1 9±1.0	1 4±0.7	1.6±0.6
20:1 18.3	1.1±0.5	1.1±0.3	1 1±0.4	1 1±0.2
20·3, 22:0	1.3±0 5	1.0±0.1	2.0±1.3	1 7±1.0
20 4 22:1	10.6±7.5	12.1±6 2	13 2±6 9	13.1±6.9
20:5 22:2	Trace	Trace	Trace	Trace
22 4	Trace	Trace	Trace	Trace

$p < 0.0$..

Table VII. *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of choline phosphoglycerides in platelets from subjects on different diets (n = 10)*

Components	Soybean oil		MCT oil	
	Before	After	Before	After
16:0	36.6±4.2	33.3±2.9	34.3±5.1	36.6±4.1
16:1	1.5±0.8	0.9±0.3	1.1±0.5	1.4±0.6
17:0	0.4±0.1	0.2±0.1	0.2±0.1	0.2±0.1
18:0	18.5±2.8	18.2±3.8	15.9±2.9	14.9±3.9
18:1	25.1±3.8	23.7±3.6	26.6±4.1	27.4±3.5
18:2	6.7±2.8	12.5±4.2	7.9±2.3	6.8±1.6
20:0	1.1±0.4	1.2±0.4	1.2±0.5	1.2±0.6
20:1, 18:3	2.3±1.3	1.9±0.5	2.9±1.0	2.2±0.9
20:3, 22:0	1.4±0.6	1.0±0.2	1.4±0.5	1.4±0.5
20:4, 22:1	7.9±1.7	6.8±2.9	8.1±3.9	8.2±4.2
20:5, 22:2	0.5±0.1	0.6±0.1	0.4±0.1	0.5±0.1
22:4	Trace	Trace	Trace	Trace
22:5	Trace	Trace	Trace	Trace

$p < 0.01$

DISCUSSION

The present study has shown that changes in the dietary fat composition during a 3-week period, in addition to changes in the quantity of the plasma lipids, induce changes of the fatty acid composition of platelet phospholipids. The changes, however, were moderate and occurred in subjects given a highly unsaturated fat diet. The medium chain, saturated fatty acids, which represented the main source of fats in the saturated fat diet did not induce significant changes in plasma or platelet lipids. The medium chain triglycerides are absorbed directly into portal blood and are almost completely oxidized to 2 carbon acetate or acetyl CoA fragments by the liver (36). As no changes of the serum triglyceride fatty acids have been observed in patients given MCT oil diet, it is not unexpected to find small or no changes in the platelet phospholipids (38).

The most consistent observation in subjects on a soybean oil diet was a relative increase of linoleic acid (18.2) in choline phosphoglycerides. This is in accordance with the observation of Cohen et al. (4), who found a high incorporation of labelled 18.2 into PC and PI of platelets compared with the other phospholipids. Phosphatidyl choline, which showed the most striking changes, also belongs to the metabolically active phospholipids in platelets according to the glycerol incorporation studies by Lewis and Majerus (18). No significant changes were observed in the levels of linoleic acid or linolenic acid metabolites (20:4, 22:4, 22:5 and 20:3, 22:5 and 22:6) in the platelet phospholipids. This may be due to the balanced content of linoleic and linolenic acid in soybean oil diet and by the rapid turnover of long chain, highly unsaturated fatty acids in platelets as shown by Hennes et al. (15) and Rahm and Holman (33). No marked decrease in any of the saturated fatty acids was found in phospholipids with increased amounts of linoleic acid. A moderate, insignificant decrease of palmitic and oleic acid usually corresponded to the increased linoleic acid content.

The changes in the fatty acid composition of the platelet phospholipids were associated with a significant reduction in activity in platelet rich plasma. Decreased PF 3 activity was also seen in platelet poor plasma. However, this decrease was less pronounced and showed greater variations from subject to subject. The availability of PF-3 after exposure of platelets to ADP and kaolin seemed mainly unaffected by the dietary changes. PF 3 activity is supposed to be caused by platelet phospholipids and lipoproteins (22). The present study indicates that the fatty acid pattern of the phospholipids is correlated to PF-3 activity in PRP, which is in accordance with the studies of Rouser and Schloredt (35). The changes in structure of the platelet phospholipids located in the membranes may reduce the PF-3 activity brought on by the mechanical strain during preparation

Table VIII. *Fatty acid distribution (g/100 g of fatty acids and aldehydes) of sphingomyeline in platelets from subjects on different diets*

Components	Soybean oil		MCT oil	
	Before	After	Before	After
16:0	23.8±1.6	21.9±4.8	21.8±3.8	21.6±3.9
16:1	0.4±0.1	0.4±0.1	0.6±0.1	0.3±0.1
17:0	Trace	Trace	Trace	Trace
18:0	4.0±2.2	7.8±2.5	5.5±1.5	7.7±2.4
18:1	3.3±2.0	5.6±3.9	3.6±2.2	4.9±3.8
18:2	Trace	Trace	Trace	Trace
20:0	3.6±1.2	4.2±2.3	4.6±1.8	4.5±1.1
22:0	27.1±6.6	25.4±4.3	26.4±5.7	21.9±3.1
22:1	9.3±5.0	4.4±[illegible]*	10.4±3.1	5.7±1.2
23:0	2.6±1.1	2.2±1.2	0.9±0.1	[illegible]±0.4
24:0	10.4±2.7	14.0±3.1	9.2±2.5	10.8±2.4
24:1	13.5±3.8	15.4±4.9	16.7±4.1	21.6±4.6

$p < 0.05$ $p < 0.01$

f PRP The moderate decrease in PF 3 activity n PRP frozen and thawed three times, from subects given soybean oil, may further indicate that he fatty acid changes, in addition to making the membrane less vulnerable to mechanical strain, also reduces the total PF 3 activity The small changes recorded probably reflect the low sensitivty of the method for discriminating between vary ing values at high concentrations of PF 3 (27).

The other platelet function tests showed no significant changes during the dietary periods. However platelet aggregation induced both by collagen and ADP showed a moderate decrease in the soybean oil group and an increase in the MCT oil group. It is evident that the method used for evaluation of platelet aggregation in the present study a method well suited for evaluation of the influence of pharmacological agents on platelet aggregation, is less suitable for the present type of studies (14). By measuring only the slope of the aggregometer curve during the initial period we obtained an expression of the lag period and the degree of aggregation per time unit. However the great individual variations made any evaluation difficult, even if each patient served as his own control.

Earlier studies have shown that increased amounts of platelet phospholipids are related to increased PF 3 activity in patients with a high frequency of occlusive vascular diseases (29 30). The present study indicates a relation also between the fatty acid composition of the platelet phospholipids and PF-3 activity However whether this represents a cause and effect relation is not definitely settled. The reduction of plasma cholesterol and phospholipids with a concomitant reduction of β-lipoproteins might also interfere with PF-3 availability as recently shown in patients with familiar hyperbetalipoproteinemia (31).

It is suggested that the benefit of unsaturated fat diets in the prevention of occlusive vascular disease is related not only to changes in plasma cholesterol levels (5 6, 17). Thrombosis often represents the final occluding event in atherosclerotic arteries and experimental studies indicate that mural thrombi may be transformed to atherosclerotic lesions (1.., ..4). Changes in platelet function caused by changes in platelet phospholipids may influence the tendency to thrombosis. Experimental studies in rats have established an inverse relation between the unsaturated fat content of the diet and the tendency to thrombosis (26 34). The present study may indicate a similar relation in man.

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AGRANULOCYTOSIS ASSOCIATED WITH AJMALIN THERAPY

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Abstract. The case reports of five patients who developed agranulocytosis while taking the antiarrhythmic drug ajmalin are presented. All five responded rapidly to withdrawal of the drug and, in four of the five, antileucocyte ajmalin-dependent activity was demonstrated in the serum by means of a leucocytotoxicity test.

Laboratory and clinical trials with the rauwolfia alkaloid ajmalin have shown it to be a valuable antiarrhythmic agent, comparable to quinidine in its actions and indications, but with apparently fewer toxic effects (1–5). Given intravenously it is effective in the treatment of paroxysmal supraventricular and ventricular tachycardias. Although the absorption is irregular and the oral route is not as reliable as parenteral injection, we have been giving this drug to selected patients orally since 1967 for the long term suppression of ectopic beats and tachycardias.

During a relatively short period we saw 3 patients who developed an agranulocytosis while receiving ajmalin, and we were given the opportunity to perform serological studies on 2 further patients admitted to another hospital. Cytotoxic, ajmalin-dependent antileucocyte activity was detected in sera from 4 of the 5 patients.

CASE REPORTS

Patient 1

A 61-year-old retired administrator was admitted in Aug. 1968 with an acute myocardial infarction. At the time he was taking para-aminosalicylic acid and isoniazid for suspected reactivation of an old pulmonary tuberculous lesion. On admission he was started on ajmalin orally 300 mg daily because of multiple ventricular extrasystoles. He was also started on acenocoumarin (Sintrom®), the thrombotest being maintained between 5 and 15%. After 3 weeks he was discharged, and shortly thereafter the tuberculostatic drugs were discontinued. On the 14th Dec. 1968 he was readmitted with a week history of sore throat, malaise and fever which had failed to respond to oral penicillin therapy. The patient denied having taken any drugs apart from the ajmalin, acenocoumarin and penicillin. On examination he was found to have a temperature of 39.4°C and his pharynx was inflamed with a small area of white exudation. The following laboratory results were obtained: Hb 14.8 g/100 ml, haematocrit 43%, leucocyte count 200/mm³ and in the smear atypical lymphocytes and monocytes without recognizable granulocytes, and platelet count 220 000/mm³. Urine examination was normal, ECG and chest X-ray unchanged in comparison with those taken 4 months previously at discharge. The ajmalin was stopped and the acenocoumarin continued. He received antibiotics (initially penicillin and streptomycin, later ampicillin). Five days after admission his temperature had fallen to normal levels, his complaints had markedly diminished and the leucocyte count had risen to 4 100/mm³ with reappearance of the granulocytes. The Hb and platelet count remained normal. He was discharged on the eighth day with a leucocyte count of 7 100/mm³ and a normal differential count. Throughout this period and subsequently he continued to receive the acenocoumarin and there has been no subsequent recurrence of the leucopenia.

Patient 2

A 73-year-old retired salesman was admitted in Feb. 1969 complaining of chest pain and shortness of breath. His ECG showed a supraventricular tachycardia of 180 beats/min. Two years previously he had been treated at home for a myocardial infarction and had been seen regularly at the outpatient department in the intervening period because of angina pectoris, for which he took pentaerythritol tetranitrate (Peritrate®). As eyeball pressure and carotid massage failed to have any effect, he was given ajmalin (50 mg i.v.) followed by a drip infusion at a rate of 1.5 mg ajmalin/min). When this failed to work, a single injection of 250 mg diphenylhydantoin i.v. was given, also without effect. He was then started on digoxin intravenously. Shortly afterwards a flutter with two to one block appeared, followed on the second day by sinus rhythm. On the eleventh day he developed multiple ventricular ectopic beats, and was started on ajmalin orally in a dosage of 600 mg daily. During the following weeks

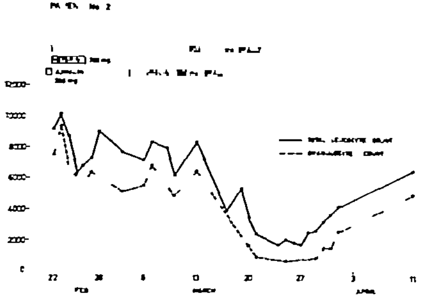


Fig 1 Patient _ Relation of drug therapy to total leucocyte count and granulocyte count.

gradual decline in th_ total leucocyte count and granulocyte count was seen (Fig. 1). The Hb and platelet count remained normal. He had no complaints at this time. Sternal puncture showed relative increase in lymphoid cells. Abnormalities in the liver function tests were seen in the form of temporarily raised alkaline phosphatase, to maximum of 5.6 Bessey units, and an aspartate transaminase to maximum of 76 Wroblewski units. The ...in was stopped, after which the leucocyte count returned to normal levels. Apart from the digoxin, which he continued to take the single dose of diphenylhydantoin, heparin during the first few days and ajmalin, he received no other drugs during his admission. The digoxin has been continued, and at subsequent follow-up examinations his blood picture has remained normal.

Patient 3

A 72-year-old man was admitted in Jan. 1969 because of complaints of palpitations and retrosternal pain with signs of mild congestive cardiac failure. The ECG demonstrated a supraventricular tachycardia with ventricular rate of 200/min. The following laboratory results were obtained on admission: Hb 14.3 g/100 ml, haematocrit 42%, leucocyte count 7 400 mm³ platelet count 110 000/mm³ blood urea 74 mg/100 ml, serum creatinine 1.4 mg/100 ml, urine albumin positive with, in the sediment, one to two pus cells, five to eight red cells and few cellular casts per high power field. The aspartate transaminase was 58 Wroblewski units and the lactic dehydrogenase 810 units with markedly raised first fraction. The chest X-ray showed slightly enlarged heart. A diagnosis of myocardial infarction with supraventricular tachycardia, and disturbed renal function possibly due to preexistent renal disease, was made.

He was given 20 mg of ajmalin i. after which h_ converted to atrial fibrillation. He was then started on ajmalin orally 300 mg daily heparin i. 300 mg daily acenocoumarin and digoxin by mouth. On the following day he reverted to sinus rhythm, and the ECG then showed signs of an anteroseptal myocardial infarction. Because of bronchial infection he was given a short course of prednisolone and tetracycline. He remained anxious about his condition and was also started on diazepam (Valium®) orally 15 mg daily. On the tenth day he developed macroscopic haematuria, the thrombotest at the time being 7% and the acenocoumarin was stopped. A microscopic haematuria, however persisted.

On the 14th day after admission he developed fever and complained of tiredness and weakness. It then appeared that he had developed agranulocytosis. All drugs were stopped—diazepam, digoxin and ajmalin. Within the following few days there was a rapid return to normal levels, the smear showing marked shift to the left" (Fig. 2). During this period the Hb decreased to minimum of 8.1 g/100 ml, the platelet count remained normal. The sternal bone marrow showed relative lack of cells except for lymphocytes. During the following week he developed an oliguria and his urea and creatinine rose from the admission levels to maximum of 336 mg 100 ml and 12 mg/100 ml respectively. T o weeks later after polyuric phase, these fell to 85 mg/100 ml and 2.1 mg 100 ml respectively.

While hyperic he again developed a supraventricular tachycardia, for which he was given quinidine orally. After further 14 days he presented with maculopapular rash, which disappeared on cessation of the quinidine therapy.

Renal biopsy performed during the oliguric phase, showed the following changes. Of the six glomeruli, one was hyaline and one ischaemic. The remaining four showed the presence of granulocytes, there was no endothelial or mesangial cell proliferation. The interstitium contained lymphocytic and plasmacytic infiltrates, and some tubular atrophy was seen. Immunofluorescence investigations showed granular anti-IgA fluorescence localised in the axial areas with sporadic membranous de

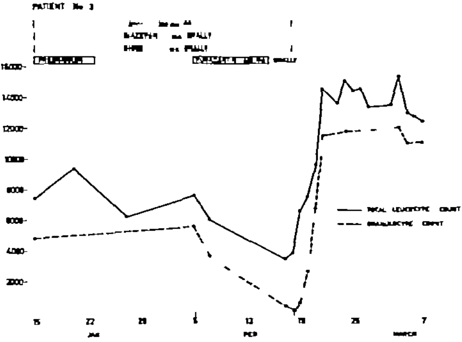


Fig. 2. Patient 3. Relation of drug therapy to total leucocyte count and granulocyte count.

poxia. Anti-IgM and anti-IgG fluorescence was present axially in one glomerulus. Fibrin and albumen were present diffusely throughout the interstitium. The findings of the renal biopsy suggested glomerulonephritis with, in addition, tubular and interstitial changes which were possibly unrelated to the glomerular process.

The patient was eventually mobilised and discharged on low protein diet as his only therapy. He has since been seen as an outpatient with recurrence of the supraventricular tachycardia. This was again treated with acenocoumarin and digoxin, without further complications in the form of haematuria, leucopenia or rashes. There has been no progression in the renal insufficiency.

Patient 4

A 76-year-old diabetic man was admitted in Dec. 1968 because of an acute anterolateral myocardial infarction. He was treated with anticoagulants (heparin and acenocoumarin) the tolbutamide and diabetic diet on which he had been prior to admission were continued. While being monitored multiple ventricular extrasystoles were seen, and he was started on 300 mg ajmalin daily. The anticoagulants were stopped shortly afterwards due to rectal bleeding from haemorrhoids. An attempt was made to stop the ajmalin when the patient was mobilized, but it had to be restarted due to the reappearance of the arrhythmia. Six weeks after the infarction he was readmitted with pyrexia and signs of pulmonary congestion. His leucocyte count was 1 700 mm³ with 18% segmented granulocytes and 6% band forms in the smear. Renal and liver function tests were normal. The patient was treated with aminophylline, digoxin and frusemide, the tolbutamide was continued and only the ajmalin was stopped. Within 2 days the temperature had returned to normal followed by an equally rapid increase in the leucocyte count and normalisation of the smear.

Patient 5

A 54-year-old man was first seen with complaints suggestive of angina pectoris in Feb. 1968. Telemetric electrocardiographic examination revealed ST segment depressions after walking approximately 350 metres, associated with many ventricular extrasystoles. He was started on acenocoumarin, 300 mg ajmalin daily and trinitroglycerine tablets during an attack. A month later he was readmitted with epilepsy for which he was treated with phenobarbitone from the fifth day. On the eighth day after this admission he developed fever with malaise, and the blood counts revealed an agranulocytosis. Only the ajmalin was stopped, and this resulted in rapid improvement in the leucocyte count and normal temperature on the second day. The phenobarbitone was continued, as was the acenocoumarin. The patient has subsequently taken trinitroglycerine without ill-effect.

SEROLOGICAL STUDIES

A leucocytotoxicity test was performed in each case; in addition complement fixation test was carried out with sera from patients 1, 2 and 3.

The leucocytotoxicity test was performed according to

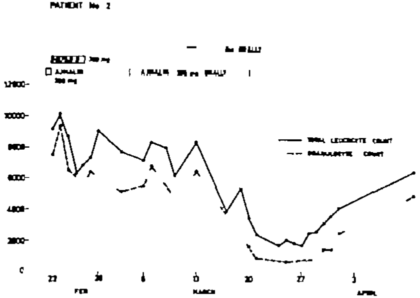


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On the 34th day after admission he developed fever and complained of tiredness and weakness. It then appeared that he had developed agranulocytosis. All drugs were stopped—diazepam, digoxin and ajmalin. Within the following few days there was a rapid return to normal levels, the smear showing a marked "shift to the left (Fig. 2). During this period the Hb decreased to minimum f 8.1 g/100 ml, the platelet count remained normal. The sternal bone marrow showed relative lack of cells except for lymphocytes. During the following week he developed an oliguria and his urea and creatinine rose from the admission levels to maximum of 336 mg/100 ml and 12 mg/100 ml respectively T o weeks later after polyuric phase, these fell to 85 mg/100 ml and 2.1 mg/100 ml respectively

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tive leucocytotoxicity test, the question arises whether the oliguric episode which coincided with the agranulocytosis might not also have been an allergic reaction. It is of interest to note that drugs which have been reported to cause an acute, presumed allergic, interstitial nephritis are often the same as may cause allergic cytopenia (6). Any conclusions which one may be tempted to draw in this case, however remain purely speculative. Although nitrofurantoin cannot be excluded as the causative agent in this patient, the literature contains few references to agranulocytosis associated with this widely used drug (2, 8).

The combination of the clinical response to withdrawal of the ajmalin medication in all of *the five patients, and the positive leucocytotoxicity* tests in four of them, strongly suggest that the ajmalin was the cause of the agranulocytosis. The agranulocytosis developed between 14 days and 16 weeks after commencing treatment with the ajmalin. As at present there is no way of distinguishing beforehand the patients who are likely to develop allergic agranulocytosis, we feel that ajmalin should preferably not be given orally for any length of time but reserved for the acute treatment of arrhythmias by parenteral administration.

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PAROXYSMAL VENTRICULAR TACHYCARDIA WITHOUT ORGANIC HEART DISEASE

A Case Report Describing its Provocation and five year Follow-up

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Abstract. This case report presents 77-year-old man with paroxysmal tachycardia, which could not be provoked by submaximal but only by a maximal exercise test. The provoked tachycardia was diagnosed as ventricular on the basis of fusion beats. During follow-up period of 5 years the patient has not had any more attacks of dizziness or syncope, and he still is without signs of organic heart disease.

A diagnosis of ventricular tachycardia does not always carry grave implications. The present report concerns an otherwise healthy young man who developed paroxysmal ventricular tachycardia on exertion, which has shown a benign course over an observation period of more than 5 years.

Case Report

A 77-year-old physical education teacher was admitted shortly after syncopal episode which occurred when he was running uphill. During the previous year he had had occasional episodes of dizziness during heavy physical exercise, especially when running, and he had sometimes noted marked palpitations in connection with the dizziness. Apart from hepatitis 5 years earlier he had otherwise been healthy previously and there was no family history of heart disease.

On admission the patient was lucid but still pale and apprehensive and had tachycardia. The ECG showed regular rhythm at rate of 230 beats/min, widened QRS complexes (0.16 sec) and no discernible P waves (Fig. 1). His B.P. was 70/50 mmHg and some minutes later—before an oesophageal ECG could be obtained—it became unmeasurable. Following metaraminol (Aramine®) i.v. and procainamide (Pronestyl®) i.v. nodal tachycardia at rate of 110/min was diagnosed, which in turn reverted to sinus rhythm within 10 min.

Subsequent physical examination revealed nothing abnormal with the exception of wide splitting of the second heart sound. The phonocardiogram showed an interval of 0.04 sec between the two components with normal respiratory variation. No murmurs were heard. Several ECGs (Fig. ?) showed neither pre-excitation nor Q–T interval prolongation, but on one occasion ventricular ectopic beats in trigeminy were recorded. A chest X-ray did not reveal any enlargement or abnormal configuration of the heart and the big vessels and the pulmonary vascularization appeared normal. Routine laboratory investigations, including thyroid, liver and renal function tests, showed nothing abnormal. Serological tests were normal except for somewhat high antistaphylysin titre, 8 U/ml, which did not increase during the period of hospitalization. Thus there was no evidence of primary or secondary heart disease.

When the patient, after 3 weeks hospitalization, developed ventricular ectopic beats in trigeminy quinidine prophylaxis was started. Following 0.. g t.d.s. for 1 day and 0.3 g t.d.s. on the next, he developed short attacks of tachycardia with widened QRS complexes. The QRS configuration during these episodes was different from that seen during the tachycardia on admission. Some hours after discontinuation of quinidine and starting of procainamide, 0.25 g six times daily the attacks of tachycardia again disappeared. The procainamide treatment was discontinued before discharge.

The ECG recorded during the attack on admission (Fig. 1) did not permit precise diagnosis of the type of tachycardia. P waves were not visible with certainty and there were no ventricular capture or fusion beats. In the subsequent ECGs premature beats with widened QRS complexes were occasionally recorded.

In an attempt to establish diagnosis of the tachycardia provocation test was performed by exercise on bicycle ergometer. Exercise of 6 min intervals and stepwise increased work loads (300 and 600 kpm/min) to heart rate of 150 beats/min was used. This procedure failed to initiate an attack of tachycardia, although

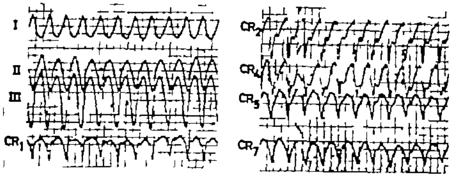


Fig. 1. ECG showing the tachycardia on admission. One square represents 0.5 mV and 0.1 sec.



Fig. 2. ECG taken some days after admission. One square represents 0.5 mV and 0.1 sec.

ventricular ectopic beats were recorded during and after the test. As the attacks were particularly prone to come on during forced running, the patient was asked to exercise to maximal work load until exhaustion. The work intensity, 1 800 kpm/min, was three times the highest submaximal load previously used. During 1 min on this load no arrhythmia developed, but during the first minute of recovery several short bouts of tachycardia appeared, all showing widened QRS complexes (Fig. 3). The QRS configuration was different from that seen during the tachycardia on admission and could not readily be compared to the QRS complexes of the tachycardia during the quinidine treatment, as different leads were employed. The exercise-provoked tachycardia showed a *distinct dissociation between P and QRS and* several fusion beats fulfilling the criteria of Marriott et al. (4). Therefore it was considered established that the provoked tachycardia was of ventricular origin.

The patient was finally discharged from the hospital without medication and he was recommended to reduce his physical training to walks only. During 5 years he has had no further attack of syncope or lightheadedness. Sometimes he feels palpitations for a short while in connection with emotional stress or physical exercise, which he has again increased. Physical examination is still normal, as is the chest X-ray. On ECGs occasional ventricular ectopic beats have been recorded. His physical working capacity is now 1 200 kpm/min at a heart rate of 170/min.

DISCUSSION

The present patient with paroxysmal ventricular tachycardia, an otherwise healthy 27-year-old man in excellent physical condition and with no known heart disease, illustrates that in this setting ventricular tachycardia may be a benign arrhythmia. During a 5-year observation period no more *syncope has occurred and repeated* cardiological examinations have not revealed any other cardiac abnormality.

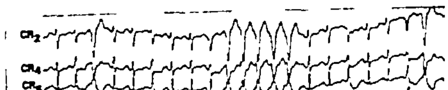


Fig. 3. ECG showing the tachycardia provoked by maximal exercise. *P*: P wave. *F*: fusion beat. One square represents 0.5 mV and 0.1 sec.

This observation seems to be in agreement with previous reports. Several cases of paroxysmal ventricular tachycardia without evidence of organic heart disease have been reported. Lesch et al. (3) collected 34 patients in the ages 2–40 years from the literature. In most patients the onset had occurred in the second and third decades. The attacks were commonly provoked by exercise, excitement and emotional stress. The symptoms were palpitations, syncope, dizziness and chest pain. Sudden death was reported for 3 of these 34 patients. In 7 of them the history of paroxysmal tachycardia was reported to be of 10 years' duration or longer the maximum being 37 years. Also Cohn et al. (1) have emphasized the benign prognosis for the transient, repetitive type of ventricular tachycardia.

Patients with relatively short attacks of tachycardia frequently present diagnostic problems. This case demonstrates that the tachycardia could be provoked by maximal exercise when submaximal exercise testing did not elicit the arrhythmia. In 10 patients of the 34 reported by Lesch et al. (3) exercise was reported as a precipitating event. The tachycardia is thus frequently related to physical exertion, but it is noted that in two of the cases in the report of Lesch et al. and in the patient of Jones and White (2) the tachycardia and/or ventricular ectopic beats could be reversed by exercise.

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DISSEMINATED TUBERCULOSIS IN ELDERLY PATIENTS

A Report on Three Cases Diagnosed intra vitam

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Abstract. Three elderly patients admitted to hospital because of suspected malignancy have been diagnosed as disseminated tuberculosis during life. Classical features of disseminated tuberculosis such as miliary mottlings on the chest roentgenogram and choroidal tuberculosis were absent in two of the patients. The difficulties hindering the establishment of the diagnosis of "cryptic" disseminated tuberculosis are emphasized by the patient records. Response to treatment with isoniazide and para-amino-salicylic acid is in some cases the only evidence of tuberculosis. Moreover, the early institution of such therapy when other evidence of tuberculosis is lacking may cure the disease before it enters an irreversible state, and is strongly recommended.

Disseminated tuberculosis may be an unexpected finding at necropsy. In two Swedish series of 2 649 and 6 606 autopsies from general hospitals during 1965–66 (3) and 1960–64 (4) the incidence of active tuberculosis was 1.1 and 1.5% respectively. Approximately one-quarter of these cases showed disseminated tuberculosis, which in most instances had not been diagnosed before death.

The difficulty in making the correct diagnosis may be attributed to several factors (1, 2, 6, 7). Recent studies have shown that the highest incidence of disseminated tuberculosis is encountered in elderly patients, often with concomitant diseases. Some of the patients only presented non-specific symptoms such as general malaise, wasting and fever. Important diagnostic clues such as miliary mottlings on the chest radiograph, meningitis and positive cultures from gastric lavages, urine and sputum might also be lacking. Under these conditions a diagnosis of disseminated tuberculosis has to be confirmed by histological features of biopsy specimens and by therapeutic trials using isoniazide and para-amino-salicylic acid. These techniques were employed in the 3 cases of disseminated tuberculosis reported on in the present paper.

CASE REPORTS

Case 1

A former plumber 69 years of age. During the past 10 years he had been treated with digitalis because of moderate heart incompensation. Since 1960 he had lumbago intermittently. He was admitted to hospital in Oct. 1968. During the preceding 6 months he had experienced progressive weakness and dyspnoea. He lost 13 kg in weight. Two weeks prior to admission he had a throat infection and the lumbago became worse. Physical examination on admission revealed a lean and pale man with slight dyspnoea. There was no oedema. The rate of the heart beats was irregular, the frequency 100 beats per min. Stethoscopy of the lungs was normal.

Hb concentration was 5.3 g/100 ml, red corpuscles 1.2 mill./mm³, platelets 130 000/mm³ and the white blood cells 500/mm³. The differential count showed an increased number of immature granulocytopoetic cells and lymphocytopenia. Serum iron was 100 μg/100 ml and TIBC 190 μg/100 ml. Haptoglobin and lactic dehydrogenase were normal. Folic acid and vitamin B_{12} were 1.1 and 656 μg/ml serum, respectively. A specimen of the sternal bone marrow showed megaloblastic erythropoesis and reactive granulocytopoesis.

The ESR was 135 mm/h. The serum protein was 6.9 g/100 ml with normal electrophoretic pattern. SGOT, SGPT and alkaline phosphatase were normal. The bromsulphalein retention was 14% after 45 min.

Repeated bacteriological analyses of blood and urine specimens were negative. X-rays of the stomach, large intestine, skeleton and lungs were normal, as were the intravenous urogram, an abdominal lymphography and cavography. The gall bladder was not visualized by oral cholangiography.

During the first 2 months of hospitalization the patient

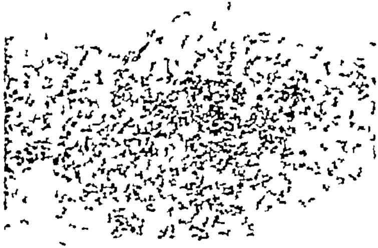


Fig. 1. Liver biopsy specimen from case 1 showing an epithelioid cell granuloma with central necrosis. May-Grünwald Giemsa stain. ×160.

deteriorated progressively and from the third month he had to be fed intravenously. The anaemia did not respond to treatment with folic acid. Pancytopenia developed and he required repeated blood transfusions. The body temperature which ranged between 37° and 38°C during the first month, later increased to about 39°C. One month after hospitalization the liver and spleen became enlarged and ascites developed. GOT, GPT and the alkaline phosphatase in serum increased above the normal range and electrophoresis of serum proteins showed reduced albumin and elevated α_2- and γ-globulin.

M. tuberculosis was not found by direct microscopy of several specimens of gastric lavages, one biopsy specimen from the liver (Menghini) and two from the sternal bone marrow. These biopsy specimens contained no granuloma. Spinal fluid had normal content of cells, protein and sugar. The tuberculin reaction was negative.

A clue to the diagnosis was obtained months after admission when an epithelioid cell granuloma with central necrosis was found in third biopsy specimen from the bone marrow. Tuberculosis was then considered as the most probable diagnosis and treatment with streptomycin, INH (isoniazide) and PAS (para-amino-salicylic acid) was instituted. Two weeks later the patient was afebrile and started to gain weight. The ascites disappeared and laboratory blood tests slowly returned to normal. At that time a new liver biopsy specimen showed epithelioid cell granuloma with central necrosis (Fig. 1). The diagnosis was later confirmed by positive guinea-pig tests and by the isolation of M. tuberculosis on Sula broth from specimens of ascites and urine. The recovery was complete and the patient is still in good health.

Case 2

A 77-year-old woman with pernicious anaemia who had been treated with hamm B_{12} during the past 4 years. She was admitted to hospital in Sept. 1968 because of weakness and fever persisting for more than 1 month. On admission a tender liver was palpable 3 cm below the rib margin. There were no signs of icterus, cardiac

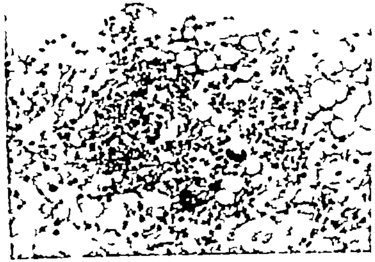


Fig. 2. Liver biopsy specimen from case showing an epithelioid cell granuloma that contains some multinuclear giant cells and is infiltrated by eosinophilic leucocytes. May-Grünwald-Giemsa stain. ×115.

insufficiency or lung disease. Morning rectal temperature ranged between 37°–38°C. GOT, GPT and alkaline phosphatase in serum were elevated, bilirubin normal. An oral cholangiography showed subnormal visualization of the gall bladder that contained several stones. The hepatic and the common ducts were normal according to the i. v. cholangiography. X-rays of heart, lungs, stomach, kidneys, colon and vertebral column were essentially normal.

During the next months the fever persisted and the patient lost 7 kg in weight. She became more tired and unwilling to leave her bed. Malignancy was suspected and the patient was transferred to Serafimerlasarettet in Jan. 1969. At that time the Hb value was 9 g/100 ml, the haematocrit 28 %, the serum iron 25 μg/100 ml and the TIBC 190 μg/100 ml. Leucocyte and platelet counts were normal. The sternal marrow showed slightly disturbed maturation of the myelopoietic cells but was otherwise normal. Alkaline phosphatase, GOT and GPT in serum were slightly elevated. Retention of bromsulphthalein after 45 min was .6 %. ESR was over 100 mm/h, serum proteins 7.1 g/100 ml, the albumin was low (2.4 g/100 ml) and the γ-globulin elevated (2.6 g/100 ml). A liver biopsy specimen (Menghini) showed granulomas with epithelioid cells, some multinuclear giant cells and diffuse infiltration of eosinophilic leucocytes (Fig. .).

Bacterial cultures from blood and urine showed no growth, whereas duodenal aspirates (obtained in the morning, the patient having fasted overnight) contained more than 10^{6} organisms per ml of two types of E. coli. Complement fixation tests for coccidioidomycosis and histoplasmosis were negative, as were cultures for M. tuberculosis on Löwenstein substrate and guinea-pig inoculations from gastric lavages.

After treatment with tetracycline (1 g/d) for 1 month was tried without clinical effects, the patient was given streptomycin, INH and PAS. Two weeks later the temperature fell towards normal values and she improved rapidly and gained weight. She left the hospital in May 1969. At that time she was afebrile. GOT, GPT and alkaline phosphatase in serum were normal, as was the liver histology. The retention of bromsulphthalein after 45 min was 12 %. On subsequent examination after almost 1 year the patient was in good condition. ESR was 48 mm/h. The bromsulphthalein retention test was slightly elevated but other liver function tests were normal.

Case 3

A 66-year-old woman, previously healthy. She was admitted to Serafimerlasarettet in Sept. 1968 of a 4 days fever and deep venous thrombosis in her left leg. On admission she was in good condition. There were no signs of heart incompensation. Physical examination of heart, lungs and abdomen was unrevealing. No enlarged lymph nodes were palpable. The thrombosis was successfully treated with heparin. The Hb value as well as platelet and white blood cell counts were normal. ESR was 66 mm/h. There was no jaundice but routine examination with liver function tests revealed high alkaline phosphatase in serum. The bromsulphthalein retention was 17 % after 45 min. Oral cholangiography showed normal gall bladder. A liver biopsy specimen (Menghini) showed some slight proliferation of the connective tissue and increased number of inflammatory cells in the liver parenchyma. No granuloma or tubercle bacilli were observed.

X-rays revealed 2 × 4 cm defect in the left part of os pelvis. A needle biopsy specimen from this tissue contained cells of adenocarcinoma type. X-ray of both lungs showed disseminated mottlings which were interpreted as lymphogenic carcinomatosis. The primary site of tumour could not be localized. Gynaecological examination as well as X-rays of the stomach, large intestine and kidneys were normal.

Three weeks after hospitalization slight fever developed. The patient became anaemic but the white cell count remained normal. A bone marrow biopsy specimen showed hyperactive myelopoiesis with an increased number of myelocytes and promyelocytes and hyperplasia of lympho-mononuclear cells. The fever persisted for the next months, the patient deteriorated and had to be fed parenterally. An abscess developed in the pelvic bone defect months after hospitalization. No bacteria were isolated on routine cultivation. At the same time a "cold" abscess appeared in the right wrist. Mycobacteria were found by direct microscopy of aspirated specimens from both abscesses. The patient became afebrile upon treatment with streptomycin, INH and PAS for 1 week and her clinical condition improved during the next 3 weeks. She then died suddenly under the picture of acute circulatory failure.

Necropsy showed miliary tuberculosis disseminated to the lungs, spleen and kidneys. The direct cause of death could not be determined.

DISCUSSION

The present patients had several features in common, i.e. high age (> 65 years), fever of long duration, weight loss, anaemia and general malaise. In all cases the onset of the disease was insidious and they had experienced their symptoms during .–9 months before admission. Two of the patients presented definite evidence of liver disease. Patient 3 had an elevated level of the alkaline phosphatase in serum but a biopsy specimen of her liver showed no signs of liver damage. Malignancy was suspected in all cases. This diagnosis could not be supported in patients 1 and ., whereas the cells of a pelvic bone biopsy specimen from patient . were erroneously interpreted as carcinomatous.

The clue to the correct diagnosis in patients 1 and . was the finding of granuloma in biopsy specimens of the bone marrow and the liver respectively. Further support for the diagnosis of tuberculosis was the successful effect of treat

ment with streptomycin, PAS and INH in all patients and the isolation of M. tuberculosis from urine, ascites and/or biopsy specimens of patients 1 and 3 Clinical characteristics of classical miliary tuberculosis, such as miliary mottlings on the chest radiogram, choroidal tubercles and tubercle bacilli in cultures of sputum, urine and cerebrospinal fluid were lacking in patient 2. This type of disease has been termed cryptic tuberculosis by Proudfoot et al. (6). Patient 1 also fulfils most of the criteria of this disease whereas patient 3 has to be classified as overt miliary tuberculosis.

The main purpose of the present communication is to recall previous reports about the difficulties of diagnosing tuberculosis in elderly patients (1 2, 6, 7) The progressive character of the disease often makes it impossible to await bacteriological confirmation of the diagnosis before start of therapy Furthermore, a therapeutic trial with INH and PAS may be the only way to diagnose the cryptic form of miliary tuberculosis (6, 7). A generous institution of such therapy is highly justified whenever tuberculosis is suspected, since this lethal disease may then be cured before it progresses to the irreversible state.

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POLYMORPHISM OF PLASMA TRIGLYCERIDE KINETICS IN NORMAL HUMAN ADULT SUBJECTS

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Abstract. Plasma triglyceride turnover has been measured in 34 adult healthy human volunteers selected on the basis of normal serum cholesterol and triglyceride concentrations and normal b.wt. The circulating triglyceride was labelled in vivo with tritiated glycerol and the disappearance of radioactivity was followed up to 24 h. The descending part of the slope was divided into exponential components with the aid of computer and the one-exponential resolutions were used for analysis. The enzymatic nature of the triglyceride removal mechanism was confirmed. When it was assumed that all subjects have common maximal removal velocity (V_{max}) level, calculation of K_m values indicated that the population under study is definitely composed at least of two and perhaps of three groups. The next analysis was therefore carried out without any presumptions and the conventional enzyme kinetic plots were drawn. These revealed that the two groups differ in regard to V_{max} but not to K_m. T of the subjects did not fit into either of these groups. Group I with higher V_{max} had significantly higher turnover rate than group II, which showed rather uniform distribution of both turnover rate and concentration. The two groups had identical age, sex and b.wt. distribution. There was definite difference in the triglyceride kinetics between males and females. Both had identical turnover rates, but females showed lower K_m and, correspondingly lower plasma concentration of triglyceride. The well known difference in plasma triglyceride concentration between men and fertile-aged women is thus accounted for by the more efficient removal system in the latter. It is concluded that the plasma triglyceride concentration in any individual and under any conditions is determined by three factors: (1) the turnover (synthesis) rate, (2) the maximal removal velocity (V_{max}), and (3) the Michaelis constant (K_m) of the removal enzyme(s). All of these may be influenced by factors like diet, hormones, metabolites and drugs, but obviously the first two are also genetically determined. It is suggested that any classification of hypertriglyceridemias should include an enzyme kinetic analysis, and this will be attempted in the studies in progress.

Since it became evident that plasma triglyceride is often elevated in patients with coronary heart disease, an extensive literature has appeared on the influences of various dietary hormonal and constitutional factors, and of disease states, on the *concentration* of plasma triglyceride. On the other hand studies concerning the turnover rate and other kinetic characteristics of plasma triglyceride are surprisingly few in number. The appearance of infused labelled FFA into plasma triglyceride has been used for estimation of the influx rate of triglyceride into plasma (3, 7, 12, 19, 20) and the turnover rate has been measured from the decay curves of labelled TGFA (13), although it is well established that these are made complex by the rapid recycling of the label (4, 6). A non-isotopic indirect method has also been described (16). Farquhar et al. (4) used glycerol-^{3}H for the in vivo labelling of plasma triglyceride and measured the turnover rate from the exponential disappearance of TG-glycerol. In fact, this method seems to be superior to the FA-labelling since the recycling of glycerol is minimal in man (4). Furthermore, it has been shown by Reaven et al. (17) that the human plasma triglyceride metabolism can be well characterized in terms of conventional enzyme kinetics. This is a particularly important finding, since, as will be indicated below, it allows a profound analysis of the whole kinetic behavior of plasma triglyceride metabolism.

In spite of its many apparent advantages the radioglycerol method has so far found only very limited clinical applications. Using this type of kinetic analysis, Reaven et al. (18) have shown that the response of plasma triglyceride turnover rate is related to average plasma insulin level during a high-carbohydrate diet, and McKenzie and Nestel (11) have characterized the triglyceride metabolism in nephrotic syndrome. None of these

Table I. *Somatic data, serum cholesterol and kinetic parameters of plasma triglyceride in male subjects*

Subject no	Age (y.)	Height (cm)	Weight (kg)	Relative weight index (kg/cm−100)	Serum cholesterol (mg/100 ml)	Plasma triglyceride: Concentration (mg/100 ml)	Plasma triglyceride: k (h⁻¹)[a]	Plasma triglyceride: Turnover rate (mg/h/kg)	Plasma triglyceride: Apparent K_m (mg/100 ml)	Plasma triglyceride: K (mg/100 ml)
1	33	187	86	0.99	226	94	0.349	15.6	75	—
2	30	178	83	1.07	239	115	0.252	13.1	131	68
3	26	176	62	0.81	185	61	0.314	8.6	138	86
4	33	176	76	1.00	229	60	0.297	8.0	150	96
5	35	188	94	1.07	192	49	0.302	6.7	156	102
6	54	170	73	1.04	278	107	0.226	10.9	168	97
7	53	170	77	1.10	243	138	0.193	12.0	184	101
8	26	175	73	0.97	177	44	0.268	5.3	189	129
9	31	190	90	1.00	213	80	0.230	8.3	190	120
10	59	175	82	1.09	173	73	0.235	7.7	193	121
11	28	175	75	1.00	229	104	0.194	9.1	216	134
12	41	170	67	0.96	243	58	0.205	5.4	243	97
13	47	177	74	0.96	248	55	0.202	5.0	253	103
14	31	174	67	0.91	176	63	0.187	5.3	270	108
15	27	180	71	0.89	220	62	0.188	5.2	272	110
16	62	184	70	0.84	289	49	0.187	4.1	286	123
17	52	175	80	1.07	239	105	0.155	7.3	298	102
18	32	177	80	1.04	258	109	0.147	7.2	315	109
Mean	39			0.99	225	79	0.[illegible]31	8.0	207	106
S.D.	±12			±0.09	±34	±28	±0.059	±3.1	±66	±16

[a] Fractional turnover rate.

reports have included data on the triglyceride kinetics in normal human subjects, however. Since we believed that any classification of hyperglyceridemic states should be made in kinetic terms rather than, e.g., on the basis of electrophoretic lipoprotein pattern, an extensive study of various hyperglyceridemias by the radioglycerol procedure was started some years ago. It soon became apparent that abnormal states cannot be characterized without a very detailed definition of the kinetic parameters in a normal material. Therefore triglyceride kinetics were determined in a number of adult human subjects selected on the basis of good health, normal serum lipid levels and normal b.wt. Analysis of the results presented here shows that the population under study is not uniform but can be separated into at least two categories on the basis of triglyceride kinetic pattern.

MATERIAL AND METHODS

The subjects were healthy volunteers from the staffs of an electric power company (Imatran Voima Oy) and a pension insurance company (Ilmarinen). They had undergone routine annual health control organized by the companies and had never been recorded to have any serious or chronic disease. They had normal B.P. and fasting blood glucose. Exclusion was made on the basis of overweight (> 1.10) and high serum cholesterol (> 290 mg/100 ml) and triglyceride levels (> 140 mg/100 ml). All women who had used oral contraceptive pills during the preceding [illegible] months were also excluded from the present series but are dealt with in [illegible] separate study. The study was not fixed to any given period of menstrual cycle. All participants were sedentary [illegible] orkers but had a variable physical activity during leisure hours. N[illegible] specific dietary restrictions were made before the study but none of the subjects admitted to having dietary habits differing from the ordinary Finnish ones. Each participant [illegible] as instructed to eat [illegible] customary home diet during the preceding day but to fast from 10 p.m. On the test day the subjects came to the metabolic ward, where they received the radioglycerol injection at 8 a.m. and stayed until evening. Fasting was continued until the 8-hour sample at 4 p.m. (total fasting time 18 h). Smoking [illegible] as prohibited but water allowed ad libitum. The final material with successful complete experiment comprises 18 men and 16 [illegible] omen (Tables I and II).

Test procedure

Glycerol-2-^{3}H with a specific activity of 200 mCi/mM was obtained either from New England Nuclear Corporation (Boston, Mass.) or from The Radiochemical Centre, Amersham, England. Originally when the preparation

Table II. *Somatic data, serum cholesterol and kinetic parameters of plasma triglyceride in female subjects*

Subject no.	Age (y.)	Height (cm)	Weight (kg)	Relative weight index (kg/cm–100)	Serum cholesterol (mg/100 ml)	Plasma triglyceride: Concentration (mg/100 ml)	k^a (h^{-1})	Turnover rate (mg/h/kg)	Apparent K_m (mg/100 ml)	K_m (mg/100 ml)
19	33	159	63	1.07	191	72	0.388	12.6	89	51
20	42	165	71	1.09	212	47	0.402	8.5	108	68
21	27	168	54	0.80	146	50	0.332	7.5	137	89
22	34	159	60	0.98	207	41	0.346	6.4	139	92
23	36	158	51	0.88	189	84	0.276	10.4	142	84
4	40	162	59	0.95	197	45	0.332	6.7	143	95
25	37	163	56	0.89	223	70	0.293	9.2	143	88
26	32	163	56	0.89	210	57	0.274	7.8	148	95
27	41	166	63	0.96	256	115	0.232	12.0	153	84
28	28	157	55	0.96	192	30	0.333	4.5	157	109
29	26	160	52	0.87	247	97	0.216	9.4	192	118
30	33	162	57	0.92	216	55	0.224	5.5	223	89
31	31	174	65	0.88	195	51	0.214	4.9	40	99
32	33	160	68	1.13	221	80	0.190	6.8	248	89
33	39	159	70	1.18	181	68	0.187	5.7	266	80
34	37	160	64	1.07	224	75	0.165	5.6	300	93
Mean	34			0.97	207	65	0.275	7.7	177	91
S.D.	±5			±0.11	±26	±22	±0.074	±2.5	±61	±12

[a] Fractional turnover rate.

ere used as such, the plasma triglyceride radioactivity decay curves were often too complex for analysis. In addition the counts obtained from a given amount of the solution were highly variable from one batch to another. As it was thought that part of the tritium might be in the form of water or other compounds, the contents of the ampoule were evaporated in vacuo, the residue was taken into 70% ethanol in saline and this solution was used for injection. Much radioactivity was lost in the evaporation process, but the remaining activity was rather constant and produced much better curves. Therefore each batch has been treated in this way before use.

A dose of radioglycerol solution containing about 4 · 10^7 cpm and diluted to 10 ml with saline was injected into a cubital vein within one min and a polythene catheter was inserted into the opposite arm. Blood samples for the determination of triglyceride radioactivity were drawn at 30-min intervals during 2 to 6 h after the injection of the label and at one-hour intervals until 10 h. The last sample was taken at 24 h. Thus, in a complete test the radioactivity-time curve was constructed from 14 points. Plasma was separated immediately by centrifugation and stored at +4°C until lipid extraction, which was carried out during the next day. Assays of plasma triglyceride concentration (9) were made from samples drawn at 0, 2, 4, 8 and 24 h.

Determination of triglyceride radioactivity

Two ml of total unfractionated plasma were extracted with 25 ml of chloroform–methanol (2:1), 5 ml of 0.5% saline was added, the tube was shaken for 5 min and left overnight at +4°C. The phases were separated by centrifugation, the lower chloroform phase was transferred to another tube and washed with 5 ml of chloroform–methanol–0.5% saline mixture (3:47:50). After centrifugation the lower phase was again taken to another tube and shaken with silicic acid. The solvent was filtered into the counting vials, PPO-toluene was added and the radioactivity was counted in a Packard Tricarb liquid scintillation counter. The separation method was checked by thin layer fractionation of the silicic-acid-treated chloroform extracts, which showed that more than 90% of the radioactivity existed in the triglyceride fraction. Thin layer chromatography of the original chloroform-methanol extract again showed that 90% or more of the plasma triglyceride radioactivity was recovered in the final solution used for counting. Thus, the total recovery of plasma triglyceride radioactivity was enough to guarantee the reliability of the method in counting the incorporation of the ^3H into plasma triglyceride.

Before adopting the above method, lipoproteins were fractionated by ultracentrifugation into density classes of <1.006, 1.006–1.019 and >1.019. The radioactivity decay curves for the two former fractions were almost identical with those of total plasma, while the radioactivity of the fraction with highest density was too small to give any reliable curves. It therefore seemed reasonable to omit the lipoprotein fractionation, particularly as this practice allowed reduction of the radioglycerol dose to minimum.

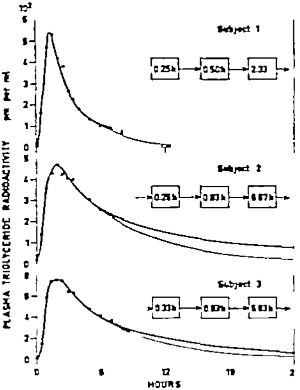


Fig. 1. Examples of Fourier transformation of plasma triglyceride radioactivity curves in three subjects with different plasma TG concentrations. The time constants shown obtained from the computer. Their order of sequence has been constructed to correspond to the physiologically most meaningful system: glycerol, liver secretory TG, and plasma TG pools.

Calculations

In order to obtain kinetic information on the pool systems which are operating in this type of experiments, Fourier transformation of the plasma triglyceride radioactivity-time curve was made in preliminary stage of the study from three unselected experiments (the cases are not included into the present normal series). The results are illustrated in Fig. 1. The curve comprises up to certain time point each of three exponential components corresponding to three pools, the time constants of which are shown in the figure. It seems reasonable to assume that these pools represent glycerol, the secretory liver triglyceride and plasma triglyceride. The removal of the label from the plasma triglyceride pool follows an exponential course between ~4 and 6–8 h, indicating that the triglyceride molecules bound to different lipoprotein fractions have identical turnover rates or if fractions with much diverging turnover rate are present, their relative amount is small. It therefore seems valid to use the exponential descending part of the curve for calculation of plasma triglyceride turnover rate. This conclusion has now been confirmed in the computer analysis of more than 400 curves.

The radioactivity-time curves were *plotted* on a semilogarithmic scale and then divided into exponential components using digital computer (8, 14). A series of resolutions were made from each curve, point by point, from the fifth point onwards. The program did not contain any presumptions concerning the number of components. The three types of resolutions, which were found acceptable to the material, are illustrated in Fig. 2, whereas the other three types recorded in Fig. 3 were regarded as unsuccessful experiments and were rejected. To give an estimate of the relative amount of acceptable and rejected experiments we collected data of 200 consecutive tests (including those presented here) without any selection and calculated the frequency of each type of success. It was revealed that 17 (8.5%) experiments were rejected, while in 111 (55.5%) instances there was a single-exponential resolution. In 57 tests (28.5%) there was a very rapid initial exponent (α_1 > 12 h^{-1}) in addition to the ordinary slope. This represented in most cases less than 5% of the total turnover and the maximal amount was 8.7%, which means that this did not influence the results to any substantial extent. This rapid exponent is in a way a computer *artefact inherent to the method and is dependent on the* random location of the first point above or below the decay curve. In addition, all experiments in which the triglyceride concentration showed clearly rising or decreasing trend were rejected because of suspicious non-steady state.

The value of mean resolution of the tracer disappearance curve was taken as fractional turnover rate (k) of total plasma triglyceride. Plasma volume was assumed to represent the distribution space of triglyceride and value of 4.5% of b.wt. was used for it. Turnover rate (V, which equals the removal rate) as mg/h/kg was calculated from the formula $V = k \cdot 0.45$ where S is the concentration of triglyceride in mg/100 ml. T

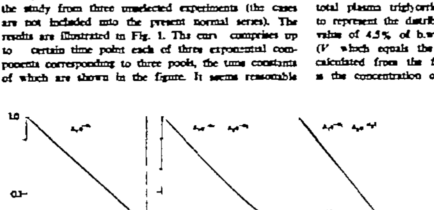


Fig. 2. Three acceptable types of computer resolution according to formula $A \ldots + A \ldots$. A = pure one-exponential component resolution. B = resolution gives two exponents, the second one being very slow and forming less than 5%. C = resolution gives two components, the first being very rapid and forming less than 5 or 10%. The zero point indicates the beginning of the analysed part of the curve.

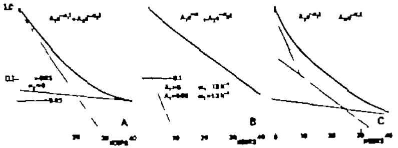


Fig. 3. Three types of computer resolution rejected from further analysis. *A* = resolution gives two exponents, one of which is very slow but forms more than 5%. *B* = resolution gives two exponents, one of which is very rapid and forms more than 5 or 10%. *C* = the curve consists of more than two components.

characterize the removal mechanisms of triglyceride from the plasma pool the results were converted to terms of enzyme kinetics. This was originally done according to the principles outlined by Reaven et al. (17). Thus it was assumed that the removal process is a saturable system with given maximum velocity (V_{max}) common to all individuals. On the basis of this assumption the Michaelis constants (K_m) were calculated from the formula

$$K_m = \frac{(V_{max} - V) \cdot S}{V}$$

The value for V_{max} was originally taken as 28 mg/h/kg, which was the highest turnover rate found in our clinical material and is also rather close to the value calculated by Reaven et al. (17) from their data. However after it became evident that the population sample studied showed distinct bimodal distribution of K_m values, the kinetic calculations were made without any presumptions concerning the constancy of either V_{max} or K_m. New values for both were then obtained from conventional plots of reaction velocity and substrate concentration (2) as illustrated under Results. The original K_m calculated from $V_{max} = 28$ has been designated as apparent K_m.

RESULTS

The basic data are recorded in Table I for males and in Table II for females. Both men and women showed a distinctly log-normal distribution of both triglyceride turnover rate and concentration. The turnover rate and concentration of plasma triglyceride were in significant correlation (Fig. 4) in both sexes. There was no significant difference in the turnover rate between male and female subjects, but the women had a lower triglyceride concentration. Thus the removal system seems to operate more efficiently in women, as is revealed also by the significantly lower apparent K_m-value. When the (apparent) K_m-values were calculated by assuming that V_{max} is similar for all subjects (17), it unexpectedly turned out that the distribution of the sample is strikingly bimodal (or probably even trimodal) in regard to K_m (Fig. 5). This mode of distribution is well evident in both sexes, although the peak of both K_m groups is slightly displaced to the left (lower K_m region) in females as compared with males. The two groups were arbitrarily designated group I and group II. It seems that the two subjects with the lowest K_m values do not belong to group I but form a separate third group, which, however has markedly lower frequency of occurrence in

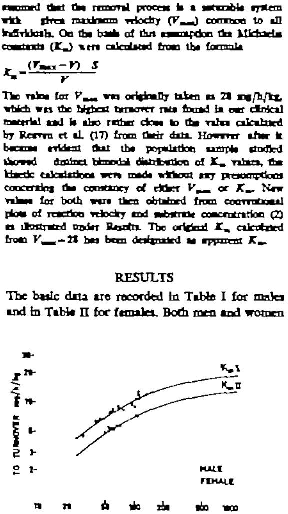


Fig. 4. Relationship of plasma triglyceride concentration and turnover rate (log scale) in the whole material. Lines corresponding to the Michaelis-Menten equation for two groups are shown (K_m I and K_m II).

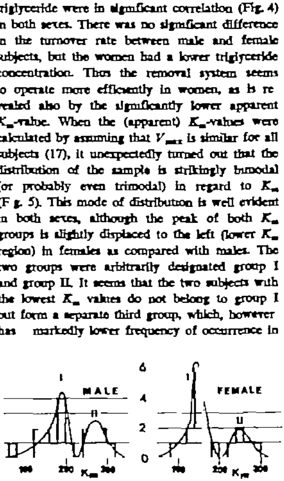


Fig. 5. Distribution of the material according to the (apparent) K_m-values calculated by assuming V_{max} value of 28.0 mg/h/kg. The vertical axis indicates the number of cases. The continuous curves have been drawn to illustrate the distinctly bimodal distribution of healthy humans and they are not based on any statistical analysis. The groups have been deliberately designated as I and II for further characterization.

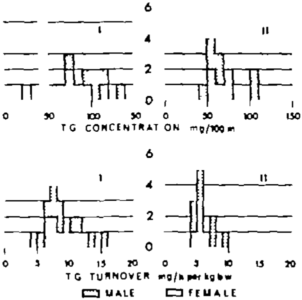


Fig 6 Distribution of triglyceride concentration and turnover within groups I and II. Note the skew distribution and wider scattering in group I as contrasted with the rather normal and uniform distribution in group II.

the population studied than the other two groups. The approximate relative frequencies of groups I and II are 0.55 and 0.40 respectively and of the third group 0.05 The two subjects (1 and 19) have been excluded from group I in the further treatment of data. On comparison of groups I and II it is evident that both plasma triglyceride concentration and turnover rate are lower and more homogenously distributed in the latter while group I seems to be much more heterogeneous (Fig. 6). Both triglyceride concentration and turnover are in a significant positive correlation to relative b wt. in group II but not in group I (Fig. 7). On the other hand, the groups do not differ with regard to age or weight distribution or sex ratio of the subjects. Age had no influence on triglyceride concentration, turnover or λ_m value.

Since it seemed plausible that, ultimately the V_{max} rather than λ_m might be the variable that separates a population into groups, it became necessary to reject the concept of constant V_{max} and to replace it by a hypothesis that the groups that had emerged in the primary kinetic analysis have a different V_{max}. To test this possibility conventional plots of $[S]$ versus $[S]/V$ and of $V/[S]$ versus V were constructed as illustrated in Figs. 8 and 9 A highly significant correlation was obtained in both groups (for $[S]$ versus $[S]/V$ $r = +0.85$ in group I and $r = +0.89$ in group II) and values for V_{max} could be calculated either from the slopes of regression lines in Fig. 8 or from the vertical axis intercepts of regression lines in Fig. 9 For the whole groups I and II the V_{max} were 20.8 mg/h/kg and 14.4 mg/h/kg, respectively Thus, normal human subjects are distributed into two major categories which have a different maximal removal capacity of plasma triglycerides. It can also be seen from Fig. 9 that

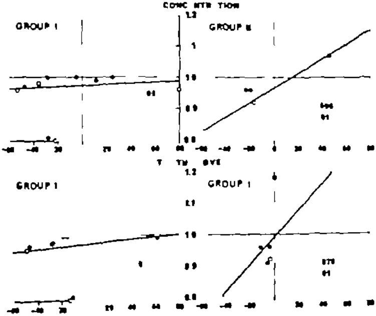


Fig 7 Plot of triglyceride concentration and turnover versus relative b.wt. in groups I and II. The scale on the abscissa is the percentile deviation from the corresponding mean calculated separately for each group and sex. The ordinate gives the relative weight index. Note the significant correlation in group II and the absence of correlation in group I. ● males O females.

the third group has probably a still higher V_{max} than group I, but due to the presence of only two points it is not possible to construct any regression line. When the new values of V_{max} are used for calculation of true Michaelis constants (given in the last column of Tables I and II) it is revealed that these are remarkably similar in both groups: mean values of 100 and 104 in groups I and II, respectively.

The above calculations are valid for the whole groups including both male and female subjects. Analysis of the data for demonstration of possible differences between the two sexes is rendered difficult by the small number of subjects remaining in each of the four subgroups. This comparison has therefore been made only within group I. The good correlation between $[S]$ and $[S]/V$ is apparent in both sexes ($r = +0.79$ for males and $+0.90$ for females). The value of V_{max} is obtained as 25.0 mg/h/kg for males and as 20.6 mg/h/kg for females. Like the apparent K_m values, the true K_m obtained from the group V_{max} levels are higher in men than in women, and this difference is still further accentuated if the K_m is calculated from the V_{max} values of each sex. This gives a mean K_m of 144 and 91 ($p < 0.01$) for men and women of group I, respectively.

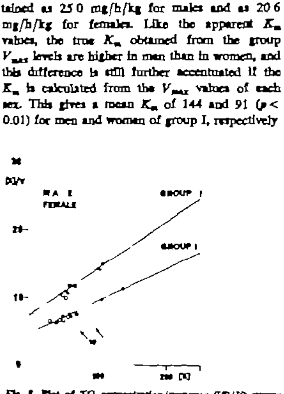


Fig. 8 Plot of TG concentration/turnover ($[S]/V$) versus TG concentration ($[S]$) done separately for groups I and II. The two subjects indicated by arrows have been excluded from the linear regression analysis. The regression equation for group I, $y = 4.77 + 0.048\ x$ and for group II, $y = 7.[illegible]4 + 0.069\ x$. The lines intercept the X-axis at a single point, which gives the value for $-K_m$ identical in both groups. The V_{max} has been calculated from the slope of the regression lines.

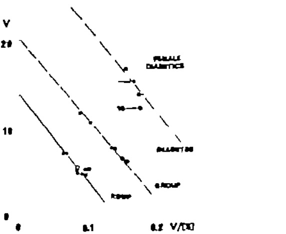


Fig. 9 To characterize the enzymatic differences of groups I and II the turnover (V) and turnover/concentration ($V/[S]$) are plotted against each other. Fixed values for V_{max} have been drawn to traverse the mean point of each group. The exact parallelism of the lines indicates the non-competitive nature of "inhibition" in group II as compared to group I. The hypothetical line for a number of diabetic subjects has been included to show the possibility of an increase of V_{max} in pathological states and to give evidence for a different nature of TG kinetics in the two normal subjects excluded from groups I and II.

Thus, in all calculations the females have a more efficient removal of triglyceride than males, even if males may have a somewhat higher maximal removal velocity.

DISCUSSION

The data obtained in kinetic analysis of a large *number of plasma triglyceride radioactivity curves* fully confirm the conclusion of Farquhar et al. (4) concerning the validity of the use of labelled glycerol as a precursor for the measurement of plasma triglyceride turnover. In the Fourier transformation the plasma triglyceride radioactivity curve was revealed to contain three components. The most rapid of these had a time constant close to that found earlier for plasma glycerol pool (15), whereas the turnover of the slowest pool well corresponds to the decay rate of plasma triglyceride (4). The intermediate pool must therefore be identical with that part of newly synthesized liver triglyceride which is on the way into plasma (secretory hepatic pool). A major part of the labelled glycerol enters the "non-secreting"

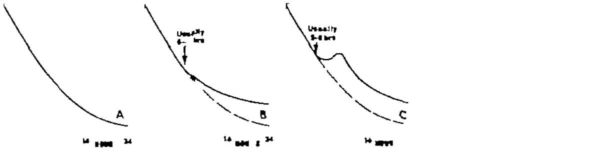


Fig. 10. Three types of curves found in successful experiments with different degrees of recycling (found in large abnormal material and not belonging to the present series of normal subjects). *A* = exponential through the whole time of analysis. *B* = continuous deviation from exponential slope at 6 to 8 h. *C* = "hump" type discontinuous deviation from exponential slope, indicating considerable pulse recycling of the label.

hepatic triglyceride pool, which has a much slower turnover. It is obvious that this pool also contributes to the synthesis of plasma triglyceride, but any quantitation of this flux by means of the kinetic analysis of plasma radioactivity curves is impossible. It is probable that a small part of the "recycling" phenomenon is accounted for by a slow influx of labelled triglyceride from this storage pool.

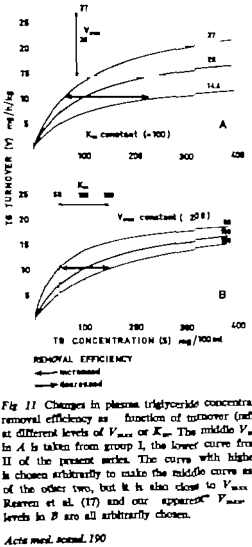


Fig. 11. Changes in plasma triglyceride concentration and removal efficiency as function of turnover (influx) rate at different levels of V_{max} or K_m. The middle V_{max} curve in *A* is taken from group I, the lower curve from group II of the present series. The curve with highest V_{max} is chosen arbitrarily to make the middle curve an average of the other two, but it is also close to V_{max} used by Reaven et al. (17) and our "apparent" V_{max}. The K_m levels in *B* are all arbitrarily chosen.

As realized by Farquhar et al. (4), some recycling of the label occurs in all cases. In our experience this phenomenon does not disturb the curve analysis, however, if the number of observations is adequate for each experiment. The three basic types of recycling observed in our series are shown in Fig. 10, all of these curves being acceptable in spite of often considerable deviation from a single-exponential decay in types B and C. In each of these model curves the part with one exponent is long enough to make the whole curve valid for a computer analysis. The curve of Fig. 10 *A* is ideal without any recycling during 24 h, while that in Fig. 10 *C* is most distorted. This latter "hump" type of curve occurs mainly in cases with a low triglyceride concentration and rapid disappearance, and it most probably indicates a rapid return flux of the label from some pool which has a time delay in its own metabolism. Liver glycogen is one possible source of this recirculation but remains so far hypothetical.

In the present procedure fractionation of lipoproteins was omitted. In our opinion this step is unnecessary and its elimination much increases the usefulness and reliability of the method without decreasing the validity of results. This conclusion can be based on the following facts: (*a*) The disappearance curve obtained for total plasma triglyceride in most instances, including also highly abnormal ones, follows closely a one exponential course, indicating the absence of

several quantitatively significant components with much different half-lives. (*b*) The turnover rates obtained for triglycerides of different plasma lipoprotein fractions are rather similar according to our own experience and to that published by Farquhar et al. (4). ( ) It has been calculated (3) from studies with labelled FFA that the total plasma triglyceride transport and that of each lipoprotein fraction agree within 5% It is important that the separation of lipoproteins can be omitted, since this makes the turnover measurement a rather simple and easy method also for clinical work.

The most significant finding of the present study is that a normal healthy human population does not show a uniform kinetic pattern of plasma triglyceride metabolism but is divided into two or perhaps three clearly separate categories with different maximal removal capacity V_{max}. This heterogeneity was not revealed in the earlier studies carried out by Reaven et al. (17 18), but their material included mainly cases with a lipid abnormality coronary heart disease or diabetes. These disorders may completely obscure the normal distribution pattern as illustrated, e.g., by the few examples of diabetes, which are presented in Fig. 9 The material of the present study was carefully selected from a normal general population by excluding all subjects with any signs of disorders that might influence the triglyceride metabolism. Apparently such normalization was necessary to uncover the basic kinetic behavior of a population sample. Since the two groups with different V_{max} showed similar b.wt., age and sex distributions, and there were no basic differences in dietary habits or physical activity between the groups either it seems justified to suggest that the difference in triglyceride kinetics is genetically determined. This statement is well consistent with the knowledge that V_{max} is related to the amount of enzyme, which, on the other hand, is controlled by gene action. More extensive genetic studies are needed, however to confirm this conclusion.

Some difficulties arise when one attempts to explain the intergroup differences other than V_{max}. While group II is rather homogeneous the members of group I are mainly responsible for the variations of both triglyceride turnover and concentration found in a healthy human population. On the other hand a correlation between relative b.wt. and triglyceride turnover was apparent only in group II. Obviously the synthesis of plasma triglyceride is more liable to fluctuate under the influence of environmental factors in group I than in group II, but the reason for this is completely obscure. The two subjects with anomalous triglyceride kinetics either have a latent disorder or belong to a third normal genetic subgroup. The only clue to the former possibility is that one of them had diabetic first degree relatives on the side of both parents.

It is clearly evident from the present results that triglyceride kinetics show a marked sex difference. The turnover rate is very similar in male and female subjects, but in every type of calculation women have lower K_m values than men. This means that at any given level of triglyceride influx women have a more efficient removal system (a higher enzyme-substrate affinity) and, accordingly a lower concentration of plasma triglyceride than men. The difference in triglyceride concentration between men and premenopausal women has been well established in several studies (1 ‑1) but never explained. It is also known that women of fertile age are fairly resistant to induction of hyperglyceridemia by a high sucrose diet (10). This finding has now a definite kinetic basis, since a low K_m and low concentration of triglyceride effectively counteract the rise of plasma triglyceride level on stimulation of influx (Fig. 11 *B*). It seems pertinent to suppose that one or more of the sex hormones act as modifiers of the removal enzymes, but whether this occurs through an inhibition by androgens or by a positive effector action of estrogens or progestagens is unknown.

In the concept outlined by Reaven et al. (17) the plasma triglyceride concentration is fixed to the triglyceride influx in a fashion that obeys the Michaelis-Menten equation. In other words, the triglyceride level is in general dependent only on the rate at which triglyceride enters the plasma, whereas the kinetic parameters of removal enzyme(s) remain constant within a species (5) and thus do not play any role in the regulation of plasma concentration. Since the results of the present study have indicated that even a normal population is heterogeneous with regard to both V_{max} and K_m of the removal system, it has become necessary to extend the basic model of triglyceride kinetics to include also the parameters

of efflux as variables. Thus the plasma triglyceride concentration is determined not only by the rate of synthesis (turnover) but also by maximal re moval velocity and K_m of removal enzyme(s) The interplay of these three factors is illustrated in Fig. 11 *A* and *B* It can be seen that the concentration is relatively insensitive to moderate changes of the rate of formation of plasma tri glycerides when this does not exceed certain upper limits and when the individual has a low K_m and a high V_{max}. On the contrary subjects with high K_m belonging to the low V_{max} group readily develop hyperglyceridemia on a relatively small increment of influx rate. For example, an increase of triglyceride synthesis rate from 8 to 12 mg/h/kg elevates the plasma concentration from 32 to 68 if K_m is 50 but from 96 to 206 with a K_m of 150 when V_{max} is 20.8. Similarly at a turnover level of 1. mg/h/kg an individual with the lowest V_{max} shown in Fig. 11 *A* develops a hyperglyceridemia of more than 500 mg/100 ml, while another with the highest V_{max} still maintains a normal plasma triglyceride concentration (80 mg/100 ml). The magnitude of increase in tri glyceride level for any given increment of influx rate ($\Delta S/\Delta V$) is thus dependent on (*a*) the starting level of turnover rate, (*b*) the V_{max}, and (*c*) K_m. If any of these parameters is basally abnormal in a proper direction, the individual will be sensitive to stimulation of plasma triglyceride synthesis, e.g. with dietary carbohydrates.

Influx and efflux rates are here considered to be independent variables even if they may be influenced by the same factors. It is well possible, however that some feedback mechanisms might operate in both directions. For example, a more efficient removal could be thought to stimulate triglyceride secretion into plasma by releasing binding sites in the circulating apolipoproteins and, on the other hand, hyperglyceridemia might induce the synthesis of removal enzyme(s) and thus increase the V_{max}.

It may be premature to predict which of the three kinetic parameters is influenced by different hereditary and environmental factors. As discussed above, the V_{max} of both synthesis and removal seems to be under genetic control but certainly can be modified also by diet and drugs. The K_m is probably regulated by hormones and metabolites and may also be one site of drug action. Apparently all three parameters are also liable to undergo changes in various diseases. The exact kinetic characterization of individual pathological states may appear difficult, however since information on the premorbid pattern is not available and, secondly both synthesis and removal processes may be altered simultaneously Through having a reference basis from a normal even if heterogeneous, population it is now possible, however to differentiate primary removal defects from cases with an abnormally high plasma triglyceride synthesis. It is to be expected that any approach to classification of disturbances in enzymatic processes should best be done in the language of enzyme kinetics.

ACKNOWLEDGEMENTS

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LEUKAEMIC SUBCLONES IN CHRONIC MYELOGENOUS LEUKAEMIA

Differences in Drug Sensitivity and in Differentiation Capacity

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Abstract. Serial karyotype studies have been done during cytostatic treatment in patient with CML in blastic crisis. A course of cytosine arabinoside and thioguanine resulted in dramatic decrease in the frequency of a 54-chromosome clone and simultaneous increases of 46, XY and 50-chromosome clone. At the same time the cytological composition of the bone marrow changed towards normal and some temporary clinical improvement took place. Comparison of data on karyotypes and cytology suggest that the highly abnormal karyotypes represented myeloblasts and promyelocytes, whereas early red cell precursors are 46, XY cells. In the discussion the following suggestions are made: (*A*) Heterogeneity of leukaemic cell population, as evidenced by differences in karyotypes, may be associated with differences in drug sensitivity. This represents rationale for multiple drug therapy. (*B*) Differentiation of leukaemic cells may not be compatible with highly abnormal karyotypes, in turn, lack of differentiation may result in growth advantage of cells with severe karyotype abnormalities, which would explain the typical evolution of CML from condition characterized by single karyotype abnormality (the Ph[1] chromosome) and successful differentiation to state with multiple karyotype abnormalities and lack of cell differentiation, ending in blastic crisis. (*C*) It might be possible to postpone the development of blastic crisis if treatment was directed against the developing abnormal clones during the chronic phase of CML. It is proposed to study the use of intermittent intensive chemotherapy in the chronic phase of CML. In preliminary observation of incipient malignant karyotype evolution in patient with CML, the secondary karyotype abnormalities almost disappeared after course of cytosine arabinoside and thioguanine.

It is well established that, in many patients in terminal blastic crisis of chronic myelogenous leukaemia (CML), marrow and blood cells comprise number of Ph[1]-positive subclones with abnormal karyotypes (2, 3, 5, 8, 11). Serial investigations have provided evidence that progression of the disease is accompanied by a karyotype evolution which leads to chromosome complements with increasing numbers of numerical abnormalities (3, 9). Besides this development of karyotype abnormalities, the late phase of CML is also characterized by an increasing lack of differentiation of marrow and blood cells, yet the possible causal relationship between the karyotype evolution and lack of cell differentiation in late CML remains to be elucidated. A mechanism to consider is that non-diploid cells differentiate less successfully than diploid Ph[1]-positive cells, in that case, an expansion of non-diploid clones at the expense of the diploid Ph -positive cell population will result in a more dedifferentiated appearance of the marrow, a decreasing production of mature, differentiated marrow cells, and consequently a deterioration of the clinical state. If this supposition were correct, and a chemotherapeutic regimen could be designed which had some selective effect upon the non-diploid clones, the result would be a shift in clone distribution accompanied by a change in the cytological composition of the bone marrow, perhaps associated with clinical improvement. The work to be reported here demonstrates that treatment with cytosine arabinoside and thioguanine in the blastic phase of CML can indeed result in marked changes in the clonal composition of the leukaemic cell population, and that these shifts may be associated with definite improvements in the cytological composition of the marrow. The fact that some highly abnormal leukaemic subclones appear to be more

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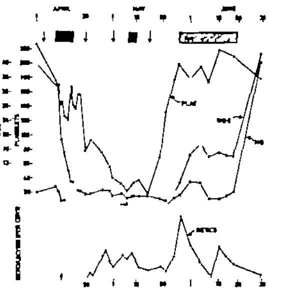


Fig 1 Effect of cytosine arabinoside and thioguanine on blood counts.
Black areas = cytosine arabinoside and thioguanine medication. Hatched area = thioguanine medication. Arrows = marrow samples. *Plat* = platelet count. *WBC* = leucocyte count. *MB* = absolute myeloblast count. *Retics* = reticulocyte count (%).

drug-sensitive than other clones raises the important clinical problem whether it will be possible to postpone the development of blastic crisis and thus increase survival time by applying intermittent intensive chemotherapy in the earlier phases of CML, i.e. when non-diploid clones begin to appear or even before.

CASE REPORT

A man, born 1899 In Jan. 1967 he was admitted to another hospital where diagnosis of CML was established, based on hepato-splenomegaly and leucocytosis of 326 000/μl with large numbers of myelocytes and promyelocytes. He was treated with splenic irradiation and from June 1967 with busulfan. He was first admitted to this hospital in Oct. 1967 at that time marked hepatomegaly and moderate splenomegaly were present. Laboratory studies: Hb 10.9 g/100 ml, platelet count 426 000/μl, leucocyte count 34 100/μl with 52% segm. neutrophils, 17% band neutrophils, 1% metamyelocytes, 16% myelocytes, 4% eosinophils, 4% basophils, 5% lymphocytes and 1% monocytes. A marrow aspirate was hypercellular dominated by neutropoietic cells. A chromosome study at that time was technically unsuccessful, but later the marrow cells were demonstrated to be Ph-positive. During the following years busulfan was given intermittently On this regimen the leucocyte count varied depending on drug administration; in periods with leucocytosis there was a moderate shift to the left in the differential count. Platelet counts were normal throughout, and the patient felt well. The Hb varied around 12 g/100 ml until Aug. 1968 then gradually declined to about 10 g/100 ml in Sept. 1969 and thereafter remained at this level. Towards the end of March 1970 the patient's general condition deteriorated rapidly: the main complaints were anorexia and severe fatigue. At readmission on April 3, 1970, moderate hepatomegaly and massive splenomegaly were present. Laboratory studies: Hb 7.9 g/100 ml, reticulocyte count 0% platelet count 175 000/μl, leucocyte count 34 400/μl with 30% segm. neutrophils, 31% band neutrophils, 5% metamyelocytes, 6% myelocytes, 14% myeloblasts, 1% eosinophils, 10% basophils, 3% lymphocytes; there was 1 normoblast per 100 white cells. The marrow aspirate was very cellular dominated by myeloblasts (51.5%) and promyelocytes (19.5%). Busulfan had been given continuously in daily doses of 2–6 mg since Oct. 1969: for 2 weeks prior to admission the dose was 4 mg daily Since the marrow now showed blastic transformation, busulfan was discontinued on April 6. From April 7 the patient received an 8-day course of 200 mg cytosine arabinoside daily and 160 mg thioguanine daily: the drugs were given 12 h apart. A 3-day course with these drugs in identical dosages was started on May 7 From May 27 to June 20, 120 mg of thioguanine was given as a single daily dose. The patient was discharged on June 20 on thioguanine 120 mg daily However he did not take this medication and reported back to the outpatient clinic on June 20 in poor condition. Arrangements for admission were made, but in the meantime his condition suddenly deteriorated further and he was taken to the emergency room at another hospital. He was dead on arrival, permission for autopsy was not granted.

MATERIAL AND METHODS

From bone marrow aspirates direct smears were immediately prepared for cytological study as described below The remaining material was injected into a bottle containing 10 ml TC 199 and 0.1 ml of a 0.04% colchicine solution. After incubation at 37°C for 1 hour the material was centrifuged and the supernatant removed. Hypotonic sodium citrate (0.95% in distilled water) was added and the specimen reincubated at 37°C for 15 min. After centrifugation and removal of the supernatant the cells were fixed twice in freshly prepared Carnoy fixative (glacial acetic acid and absolute methyl alcohol 1 3) and left in the fixative for one hour at −20°C. The cells were centrifuged again, fresh fixative was added and the metaphases were spread by evaporation of the fixative over flame. The slides were then stained with Giemsa stain. Fifty well spread metaphases from each marrow sample were karyotyped under direct observation in the microscope. The metaphase figures suited for analysis represented a varying minority of the total metaphase figures.

The direct marrow smears were stained with Leishman stain, and from each specimen differential count of 100 consecutive red and white cell precursor mitoses in pro- or metaphase was carried out. A the marrow for

karyotype analysis was incubated with colchicine for an hour before the hypotonic treatment, the karyotyped metaphases must have included mitoses in pro- or metaphase at the time of sampling but not those in ana- or telophase. Consequently ana- and telophase mitoses were excluded from the differential counts of mitotic figures.

Mitoses with an agranular, clear basophilic cytoplasm were classified as myeloblast mitoses. If the cytoplasmic granulation was purely or predominantly azurophilic, the cells were scored as promyelocytic. Mitoses were classified as myelocytic if they displayed predominantly specific granulation. Of erythroid mitoses, only macronormoblast and pronormoblast (L3) mitoses were included; mitotic figures of later (smaller) erythroid precursors were disregarded since it was noted that small metaphase figures in the chromosome preparation were not sufficiently well spread to be analysed.

Differential counts of interphase cells were based on 200 cells.

RESULTS

Blood and marrow cytology

Fig. 1 shows the blood counts during the period of study. In brief, cytosine arabinoside and thioguanine were followed by a severe drop in the leucocyte count with virtual disappearance of blast cells from the blood, temporary thrombocytopenia, and resumption of effective erythropoiesis as judged from the reticulocyte count. Tables I and II show the cytological distribution

Table I. *Differential counts of bone marrow cells (%). Cytosine arabinoside + thioguanine given from April 7 to April 14 and from May 7 to M y 9*

Cell type	Date 3/4/70	20/4/70	1/5/70	15/5/70
Myeloblasts	51.5	3.5	7.5	11.5
Promyelocytes	19.5	3.5	16	11
N. myelocytes	8	15.5	7	7
N. metamyelocytes	1	14.5	1.5	4.5
Band neutrophils	5.5	16.5	3.5	13
Segm. neutrophils	5.5	22	21	16
Eosinophils	3.5	5	7	6
Basophils	4	11	6	2
Macronormoblasts	0	0.5	1	0
Pronormoblasts	0	1.5	1	0.5
Basophilic normoblasts	0.5	3	1.5	1
Early polychromatic normoblasts	0	2	4.5	5.5
Late polychromatic normoblasts	0	0.5	13	15.5
Lymphocytes	1	1	9.5	6.5
Cellularity	+++	+++	+	+

Table I. *A*... *from* ...

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Fig. 3. Karyogram of the 46 XY, G(Ph[1])+, C(?) ... line

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Table III. *Chromosome count distribution in the bone marrow samples (cf text to Table I)*

Date	43	44	45	46	47	48	49	50	51	52	53	54	55	56	Total
3/4/70	0	0	0	1	1	0	1	1	2	1	8	34	0	1	50
20/4/70	1	0	2	10	1	0	4	18	0	2	1	10	1	0	50
1/5/70	0	0	2	12	1	2	4	20	0	1	1	6	1	0	50
15/5/70	0	0	2	14	0	0	4	27	2	0	0	1	0	0	50

Table IV *Clone frequency of all metaphases (%). The 46 XY cells all contained one Ph¹ chromosome*

	Date			
Clone	3/4/70	20/4/70	1/5/70	15/5/70
54, XY G (Ph¹)+ F+ C(5)+ A_3+	50	12	4	2
50 XY G(Ph¹)+ C(3)+	0	22	30	48
46, XY	0	16	12	26

chromosomes showed a roughly even distribution over the various chromosome groups.

Fig. 6 shows the percentage of metaphases in the various samples with supernumerary Ph¹ F C and A_3 chromosomes. It appears that cells with additional F and A_3 members were the most sensitive to therapy a precipitous decline in their frequency was observed from sample 1 to 2 this decrease continued even further during the time from sample 2 to 3 when no cytostatic drugs were administered. Cells with supernumerary Ph¹ and C chromosomes also decreased, although to a lesser degree during treatment periods, but increased between samples 2 and 3 when therapy was withheld. It should be noted that the data of C+ karyotypes shown in Fig. 6 represent the pooled frequencies of metaphases with additional C members. The individual cells, however presented *widely varying numbers of extra* C chromosomes, and the frequencies of the corresponding karyotypes showed different patterns of variation from sample to sample. These variations appear from Fig. 7 The most pronounced changes of prevalence occurred in metaphases with 3 and 5 supernumerary C chromosomes, respectively Whereas cells with 3 supernumerary C chromosomes (C(3)+ cells) show a steep rise preferably in connection with chemotherapy the C(5)+ cells exhibit a rapid and continuous fall. In contrast, the frequency of C(2)+ and *C(4)+* metaphases is relatively constant. C(1)+ and C(6)+ karyotypes were also found, but only in 0–2 cells in each sample.

Most of the metaphases containing F+ C(5)+ or A_3+ (Figs. 6 and 7) members belonged to the 54-chromosome clone (Fig. 5) Correspondingly

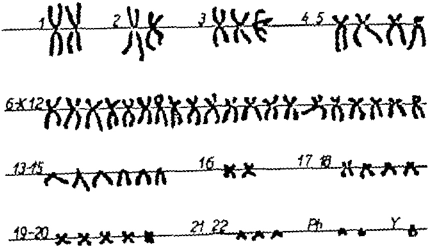


Fig 2 Karyogram of the 54, XY G(Ph¹)+ F+ C(5)+ A_3+ clone.

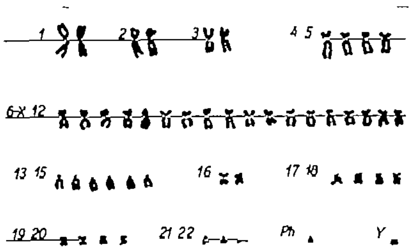


Fig. 3 Karyogram of the 50, XY G(Ph[1])+ C(3)+ clone.

the frequency of these individual components rather closely paralleled the prevalence of the 54-chromosome clone. It cannot be decided whether the drug sensitivity of this clone was connected with the aggregate of all the karyotype abnormalities or with a particular abnormality. It is improbable, however, that the sensitivity was linked with the extra Ph[1], since an extra Ph[1] was also present in the expanding 50, XY, G(Ph[1])+ C(3)+ clone. The success of this latter clone appears to be connected with the combination of C(3)+ with an extra Ph: thus karyotypes with C(3)+ but no additional Ph[1] showed a less definite increase (2, 12, 8 and 14% respectively in samples 1–4).

Comparison of karyotype and cytological data

Fig. 8 shows a comparison between the variations of certain karyotypes and of cytologically classified mitotic figures. It appears that the frequencies of Ph[1]-disomic metaphases run a course similar to that of the sum of myeloblast and promyelocyte mitoses. This similarity may suggest that the majority of the Ph[1]-disomic karyotypes originated from myeloblasts and promyelocytes. Similarly the frequencies of myeloblast mitoses roughly follow those of the 54-chromosome clone, which may indicate that cells with this highly abnormal karyotype were preferably or exclusively myeloblasts. On the other hand, in

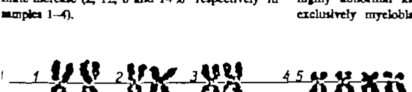


Fig. 4 Karyogram of the Ph[1]-positive 46, XY clone.

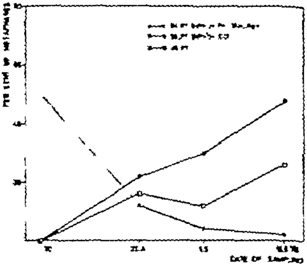


Fig. 5 The three main clones in % of the total numbers of karyotyped metaphases.

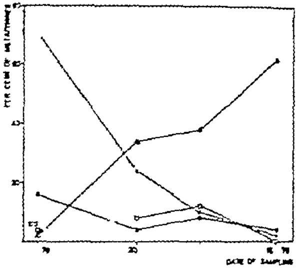


Fig. 7 Karyotypes with different numbers of supernumerary C chromosomes in % of analysed metaphases. The frequencies of C+ and C(6)+ karyotypes varied between 0 and 4%.

samples 3 and 4, most of the myeloblasts seem to have had other karyotypes.

In Fig. 9 the frequency of "diploid" Ph positive karyotypes is compared with the frequency of myelocyte and early red cell precursor mitoses. There seems to be good agreement between the frequency of the red cell precursor mitoses which were assumed to be karyotypable (i.e. excluding the small mitotic figures) and the diploid metaphase frequency. No such relationship is apparent for myelocyte mitoses.

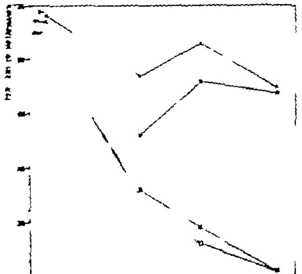


Fig. 6 Frequencies of karyotypes with supernumerary Ph[1] F C, and A_1 chromosomes.

DISCUSSION

The karyotype data clearly demonstrate the heterogeneity of the leukaemic cell population. The non-uniformity of the leukaemic cells also expressed itself in their different response to therapy. Due to the presence of three major cell clones it was possible to trace the substantial variations in clonal composition which took place as a result of treatment. Following cytosine arabinoside and thioguanine there was a dramatic decrease of the 54-chromosome marrow clone and a corresponding rise of a diploid and a 50-chromosome clone. This must reflect a particular drug sensitivity of the 54-chromosome clone: the alternative possibility, i.e. that the 54-chromosome clone could have been "hidden" by an absolute increase of the more successful clones, is unlikely since the marrow cellularity, as judged from the cell density in the smears, did not increase between the first and the second marrow sample and later rather decreased.

It is to be noted that in the first marrow sample none of the later numerous 50, XY, G(Ph$_1$)−, C(3)− and +6, XY (containing 1 Ph[1]-chromosome) karyotypes were observed. There could be

two reasons for this: 1) These cells were in a dormant state at the time of the first sampling; in that case they would not show up in karyotype analyses and, by virtue of being in a non-cycling state, they would be insensitive to chemotherapy. Eradication of the large 54-chromosome clone might elicit a feedback mechanism which would trigger the dormant cells into proliferation (6). 2) These cells did proliferate at the first time of sampling but represented such a small fraction of the proliferating cells that they were not encountered in an analysis of 50 karyotypes. In this case these cells must, for some reason, have been relatively drug-resistant; that they were rather resistant even when in an actively proliferating state is suggested by their increase from sample 3 to sample 4, during which interval chemotherapy had been applied. Whatever the mechanism of the relative drug resistance of the 46, XY and the 50-chromosome clones, the data show that when a diversity of leukaemic cells exists, eradication of an abnormal clone may enable other leukaemic clones, rather than normal cells, *to expand. This appears to be a strong* argument for employing multiple drug therapy

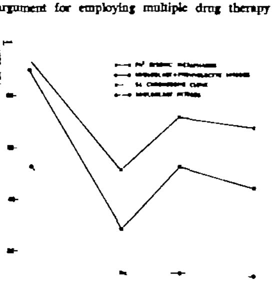


Fig. 8. Comparison of karyotypes and cytological data on mitotic figures. The ordinate indicates the percentage of all karyotypes that belong to particular clone and the percentage of mitotic figures that belong to particular cell type. Ph¹-disomic metaphases parallel the sum of myeloblast + promyelocyte mitotic figures. The 54-chromosome clone parallels myeloblast mitotic figures.

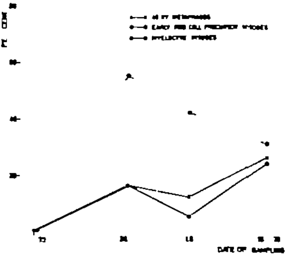


Fig. 9. Comparison of karyotypes and cytological data on mitotic figures. Ordinate as in Fig. 8. The 46, XY metaphases, which contained one Ph¹-chromosome, parallel the early red cell precursor mitotic figures.

either simultaneously or in rapid sequence, in an attempt to suppress leukaemic growth in toto.

Another aspect of the present data is the possible relationship between clone prevalence and the cytological composition of the bone marrow. An impression of whether a certain clone is associated with a particular type of cell can be gained by comparing the percentage of all karyotypes represented by a certain clone with the distribution of mitotic figures in respect of cell types in the panoptically stained marrow smears. It must be recognized that no definite conclusions can be drawn from this approach. The reason is that only a minority of karyotype figures are sufficiently well spread to allow the complete analysis which is necessary to assign them to a particular clone. Hence, comparisons between differential counts of mitotic figures and clone frequencies rest on the assumption that the analysable karyotypes represent an unbiased sample of all karyotypes. However, the evidence for identity of karyotype and cytological type is strengthened when, as in this study, parallel variations in clone prevalence and in cell type prevalence among mitotic figures are observed in sequential bone marrow samples.

The 54-chromosome clone almost certainly accounted for most of the myeloblasts before therapy. This is supported by the coincident marked

decline in the 54-chromosome clone prevalence and the decrease in mitotic and interphase myeloblasts in marrow and blood. The agreement between the frequencies of Ph¹-disomic metaphases and those of myeloblast + promyelocyte mitoses (Fig. 8) suggests that a majority of the immature cells contained two Ph¹-chromosomes. One may assume that cells with these karyotype abnormalities were so severely disturbed that they were unable to differentiate beyond the myeloblast-promyelocyte level whether the cause of this was the abnormal karyotype can, of course, not be decided.

Conversely there is an association (Fig. 9) between the frequency of red cell precursor mitoses and (Ph¹-positive) 46 XY metaphases, i.e. the karyotype typical of the chronic phase of CML. This suggests that erythroid differentiation was possible only in cells which did not have highly abnormal karyotypes. In this context it is of interest to note an observation by Müller (7): in two cases of CML in blastic crisis, the marrow cells had a hypodiploid DNA-content mode in one case and a hyperdiploid mode in the other: in both cases, however the segmented granulocytes in the blood were diploid. This demonstrates that only diploid precursor cells succeeded in producing cytologically fully differentiated cells. In the red cell series there is some suggestive evidence that the differentiation product, haemoglobin, exerts a negative feedback effect on proliferation (12). Extrapolating from this, one may speculate that the differentiation block which appears to be associated with some karyotypes may enable these particular cell lines to undergo a higher number of consecutive divisions than the four consecutive divisions which take place in normal differentiating granulocytopoiesis (1). Ceteris paribus, this would lead to increased proportions of immature cells and abnormal karyotypes in the marrow. At least in part, such a mechanism could explain the usual development of CML from a condition characterized by differentiating cells and a single chromosome anomaly to a state dominated by poorly differentiated cells with many karyotype abnormalities.

However whatever the mechanism may be, it is clear that the main threat to the patient with CML is the development of blastic crisis. In a way CML may be considered as a highly characteristic and therefore diagnosable preleukaemic condition which has a very high probability of developing into acute myeloid leukaemia (the blastic crisis). In the management of the chronic phase of CML the predominant method is to apply a single agent (busulfan) in order to maintain the blood counts at acceptable levels. This regimen keeps the patients in a state of well-being, but has little effect on survival time (4). In order to prolong survival time, it will be important either to increase the efficacy of treatment in blastic crisis, or preferably to postpone the development of the blastic crisis. Conceivably this could be done by giving, in addition to busulfan, intermittent courses of multiple drug chemotherapy during the chronic phase in order to set back the abnormal clones. In this context it is important to note that abnormal karyotypes appear long before the clinically apparent blastic transformation (3 10 11). Whether such treatment will be of value can only be decided from clinical trials. However some preliminary information on the possible rationale of such a regimen may be gained from sequential karyotype studies in relation to intermittent courses of chemotherapy. We have recently observed a patient with previously untreated CML who did not respond optimally to busulfan. After 6 months' therapy there was still slight anaemia, the splenomegaly had not regressed, and the differential count still showed a shift to the left in spite of a normal leucocyte count. Malignant karyotype evolution was suspected, and this was confirmed in a study done on April 13 1970: out of 50 metaphases 13 had pseudodiploid karyotypes, hyperdiploid cells were not observed. Starting the same day a 6-day course of cytosine arabinoside and thioguanine was given. On May 13 1970, karyotype analysis was repeated. out of 47 metaphases, only one was pseudodiploid. Compared with the pretreatment values, this decrease is significant at the 0.005 level. At the same time there was a transitory improvement in the clinical parameters.

Although, so far this is a single observation, it appears encouraging and suggests that this approach should be further studied.

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IMMUNOGLOBULIN STUDIES IN GASTROINTESTINAL DYSFUNCTION WITH SPECIAL REFERENCE TO IgA DEFICIENCY

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Abstract. The concentrations of the five immunoglobulins G, A, M, D and E have been studied in 34 adults with gastrointestinal dysfunction. Four patients lacked detectable amounts of IgA as determined by gel precipitation methods. The mean levels of the other four immunoglobulins were practically normal. It is doubtful whether there is any relation between IgA deficiency and gastrointestinal dysfunction.

During recent years several reports have been published concerning immunological abnormalities in malabsorption syndromes, and the possible role of IgA has been especially considered. The greatest importance of IgA is believed to be in the defence against infections of the mucosae of the respiratory and intestinal tracts.

The present report concerns the serum concentrations of immunoglobulins G, A, M, D and E in patients with intestinal dysfunction. Four of the total of 34 patients studied had no detectable IgA. We have stressed the uncertainty with which IgA deficiency can as yet be related to gastrointestinal dysfunction and, especially the question of a causal relationship, which is discussed in detail.

In order to shed light on the possible role of immediate allergic reactions in gastrointestinal dysfunction, the serum concentration of IgE was determined. This is of especial interest since it has been shown that a considerable proportion of the IgE production occurs locally in the mucosa of several organs (13)

MATERIAL AND METHODS

The study comprised 34 patients, 9 men and 25 women, who had been investigated during the last 4 years at the Department of Medicine, University Hospital, Uppsala, for symptoms of gastrointestinal dysfunction, mainly diarrhoea. Four patients were found to lack detectable concentrations of IgA in their serum, and their case histories are given.

Samples of venous blood were collected and, after clotting, the serum was withdrawn and stored at −20°C until analysed. Saliva and urine samples were concentrated about 50 times by ultrafiltration prior to analysis, carried out by Professor L. Hanson, Gothenburg

Serum protein analyses by zone electrophoresis on filter paper and agarose gel were performed at the Department of Clinical Chemistry University Hospital, Uppsala.

Quantitative estimations of the immunoglobulins G, A, M and D were made by single radial diffusion in gel according to the method of Mancini et al. (18), with some modifications (16). The IgE concentrations were determined by the radio-immunosorbent technique (RIST) (15), and the quantitative estimation of disaccharidase activity was determined at the University Hospital, Lund, by method described by Dahlqvist (6)

CASE REPORTS

Case 1

A 42-year-old woman, Para 0, who had had the usual childhood diseases as well as diphtheria. She had always been readily susceptible to colds and ear infections. From the middle of the 1940s up to 1960 she had pneumonia at least once year. In childhood she was also hospitalized for congenital ankylosis of several vertebrae. At 10 years of age she was treated for short period of intestinal catarrh. At the time of the present study she still had sensitive stomach and slight diarrhoea in association with her upper respiratory infections, which occurred about once monthly. She had been receiving monthly injections of gammaglobulin for several years.

Physical examination. A small, slender woman with thoracic kyphosis and short neck. BP 130/100 mmHg. Heart and lungs normal on auscultation. Palpation of abdomen and rectal canal revealed nothing abnormal part from superficial ring of small haemorrhoids. Physical status otherwise normal.

Table I. *Immunoglobulin concentrations in serum from patients with gastrointestinal dysfunction, with special reference to 4 patients lacking IgA*

	IgG mg/100 ml	IgA mg/100 ml	IgM mg/100 ml	IgD mg/100 ml	IgE ng/ml
Healthy adults[a]	1 323	158	88	4.9	248
Whole series (34 pat.)	1 323	168	131	2.3	112
Case 1	160	1	18	1	11
Case 2	440	1	10	1	12
Case 3	2 290	1	390	1	73
Case 4	2 200	1	410	1	153

From Johansson 1968 (11) and Johansson et al. 1968 (16)
[a] Two patients (cases 1 and 2) with hypogammaglobulinaemia are not included in the calculations. One patient (case 3) was receiving gammaglobulin intramuscularly and is not included in the IgG mean value. In the calculations an arbitrary value of 0.5 mg/100 ml was used when no IgA or IgD could be detected.

Laboratory data. Hb 12.5 g%. WBC, differential white blood cell count, and thrombocyte count, all normal. ESR 1 mm/h. Sternal puncture showed normal erythro- and myelopoiesis, but no plasma cells were found. The plasma electrolytes, creatinine, cholesterol, uric acid, alkaline phosphatases, bilirubin and transaminases (GOT GPT) in serum were all normal, as also was the thrombotest value. Concentrations of folic acid and vitamin B_{12} in serum normal. No L.E. cells were found. The latex fixation test for rheumatoid arthritis gave negative result, the antistreptolysin titre was below 64 U/ml and the antistaphylolysin titre 0.125 U/ml. Complement fixation tests for adenoviruses and polioviruses I–III gave titres below / Neutralization test for antibodies against Coxsackie viruses 1 5 showed a titre below /. The Mantoux reaction and the Meinicke and Kline reactions were negative.

The concentrations of the serum proteins are shown in Table II and those of the serum immunoglobulins in Table I.

The IgA level in saliva was below 10 μg/ml, and there were no detectable immunoglobulins (less than 1 μg/ml) in the urine.

Gastrointestinal tract. Roentgenography of the large intestine, stomach and small intestine showed normal conditions, as also did sigmoidoscopy. A biopsy taken from the jejunum showed slight but distinct pathological changes consisting of an oedematous loosening of some villi, dilatation of lymphatic vessels and considerable lympholeukocytic infiltration of the mucosa. Plasma cells were found in normal quantities. The changes resembled those in exudative or protein-losing enteropathy (Fig. 1).

Quantitative analysis of the disaccharidase activity in the jejunal biopsy specimen showed normal values (Table III).

Oral glucose, maltose and lactose tolerance tests showed normal increase of the blood sugar values. A fructose tolerance test gave a rather flat curve but not definitely pathological. In an ral xylose tolerance test 26% of the given dose was excreted in the urine. No diarrhoea occurred in association with the carbohydrate loading, but xylose loading was followed by several loose stools.

Faecal cultures showed a growth of normal enteric flora. The stools contained no blood.

Respiratory tract. Roentgenograms of the lungs were quite normal. In the sputum a growth of Staphylococcus aureus resistant to sulphonamides and penicillin was found.

Course. The patient was still continuing the previous treatment with injections of 16 ml of gammaglobulin solution every month t the time of the last follow-up. Gammaglobulin (Porlet) known to contain consider able amount of IgA (Johansson, unpublished observation) was also given for 3 months but with no effect. The upper respiratory infections were still occurring approximately once a month at the last follow-up and were accompanied by few days of loose stools.

Case 2

A 57-year-old woman, gravida I, Para 0, ho had had pneumonia at least four times before the age of 10 years. Further ttacks of pneumonia in 1930 19_3 1957 1960 and 1966. Since the end of the 1950s she had also had an increased susceptibility to upper respiratory infections, sometimes complicated by sinusitis. In 1946 she was treated at her local hospital for intestinal catarrh with diarrhoea, and roentgenological examination of the large intestine at that time was said to have shown signs of inflammation.

She was now admitted for investigation for eight

Table II. *Electrophoretic analyses of sera from 4 patients with gastrointestinal dysfunction and lacking IgA. The concentrations are given in g/100 ml*

	Total protein	Albumin	Globulins α_1	α_2	β_1	β_2	γ
Case 1	6.0	3 7	0.3	0.6	0.5	0.4	0.4
Case 2	4.8	3.0	0.4	0.3	0.3	0.3	0.5
Case 3	8.1	4 0	0.3	0.6	0.4	0.4	2.4
Case 4	7.9	4 1	0.3	0.6	0 5	0.4	2.1
Healthy adults	6.3–7.9	3.4–4 6	0.2–0.6	0 4–0.7	0.4–0.7	0.2–0.6	0 8–1.4



Fig. 1. Jejunal biopsy from case 1 showing oedematous loosening of the villi, dilatation of lymphatic vessels and considerable lympholeukocytic infiltration of the mucosa.

loss f 10 kg during the last year and continuing and diarrhoea especially t night. No blood noticed in the stools. She had been having about seven loose stools in 24 hours.

Physical examination. A thin, slight woman with signs of muscular atrophy. Reduced hair in the axillae and pubic region. No paleness. B P 130/85 mmHg. Heart and lungs normal on auscultation. Abdominal and rectal palpation revealed nothing abnormal. General neurological examination showed normal conditions.

Laboratory data. Hb 113. Differential white blood cell count and thrombocyte count normal. ESR 4 mm/h. Plasma electrolytes, creatinine, plasma lipids, protein-bound iodine, alkaline phosphatases, bilirubin and transaminases in serum were all within normal values. Serum folic acid and itamin B_{12} also normal. The concentrations of the serum proteins are shown in Table II and those of the immunoglobulins in Table I.

The Mantoux reaction as negative, and neutralization antibodies against poliovirus type 1 ere present in a titre of /. Complement fixation antibodies against adenoviruses ere found in titre of / and against Coxsackie B 1–5 in a titre of /. Widal reaction and the Meinicke and Kline reactions were negative.

Gastrointestinal tract. Roentgenograms of the stomach and small intestine were normal. The sigmoid and descending colon and the left side of the transverse colon showed haustral irregularities. No stenotic changes wer observed. Sigmoidoscopy up to 20 cm showed generally rather red mucosa with no ulceration. No suspicion of tumour. No bleeding. Jejunal biopsy showed moderate atrophy of the villi with leukocytic infiltration (Fig. ?)

The excretion of faecal fat was within the normal range. Quantitative analysis of the carbohydrate enzyme activity in the small intestine showed low values throughout (Table III), indicating general disturbance of the mucosa of the small intestine, and the picture resembled that in gluten-sensitive steatorrhoea.

Oral maltose and glucose tolerance tests gave normal results, but there was considerable diarrhoeal reaction to loading with lactose and fructose. Hardly any increase of the blood sugar occurred in these latter tests. On xylose loading ther was urinary excretion of only 11% of the given dose.



Fig. Jejunal biopsy from case showing moderate atrophy of villi and leukocytic infiltration.

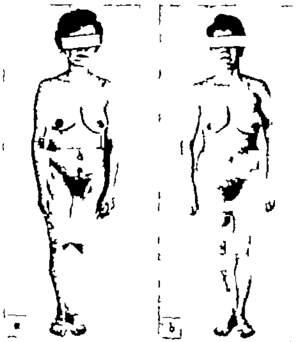


Fig 3 (*a* and *b*). Cases 3 and 4 (uniovular twins). Note the difference in the subcutaneous fat layer. Case 4 had only mild intestinal symptoms.

The total acidity of the gastric juice was 55 mEq/l and the free HCl 40 mEq/l. Faecal cultures showed growth of Pyocyaneus and Staphylococcus aureus, both sensitive to sulphonamides. The stools contained no blood.

Respiratory tract. Staphylococcus aureus and α-streptococci were cultured from the sputum. Roentgenograms of the lungs showed slight shadowing in the right middle lobe.

Course. The diarrhoea disappeared completely after a period of monthly injections of gammaglobulin, but despite continuation of the injections it recurred sporadically during the 3 years up to the time of the present study.

Case 3

A 39-year-old woman, gravida IV and Para IV who had had the usual childhood diseases but had not been particularly susceptible to infections. At the age of 25 years she was treated with iodine for goitre on tw occasions. In 1963 she underwent cholecystectomy and in 1964 and 1965 she had pneumonia. Since 1965 she had suffered considerably from diarrhoea and had lost 7 kg in weight. She also had suffered from gastritis-like symptoms periodically. She was investigated for the first time in 1967 when gastrointestinal roentgenograms were normal. Sigmoidoscopy also showed essentially normal conditions. The excretion of fat in the faeces was within normal limits. Widal' reaction was positive but repeated cultures from the faeces showed no growth of Shigella or Salmonella bacteria. An abundant growth of fungi of the Candida albicans type was found in the faeces, how ever. There was some improvement after medication with Salazopyrin® was begun, but she was readmitted for further investigation.

Physical examination. A small, slenderly built, thin woman. No signs of peripheral decompensation. BP 130/90 mmHg. Heart and lungs normal on auscultation. Abdominal and rectal palpation revealed nothing pathological. Physical status also normal in other respects.

Laboratory data. Hb, WBC, differential white blood cell count and thrombocyte count all normal. ESR 30 mm/h. Plasma electrolytes, creatinine, bilirubin, alkaline phosphatases and transaminases in serum all within normal limits. The serum concentrations of vitamin B_{12} and folic acid were normal.

The concentrations of the serum proteins are shown in Table II and those of the immunoglobulins in Table I.

The Mantoux reaction was positive and the Meinicke and Kline reactions negative. This time Widal's reaction was negative. No L.E. cells were found, and the latex fixation test for rheumatoid arthritis gave negative result. The antistreptolysin titr in serum was below 70 U/ml and the antistaphylolysin titre 0.12 U ml.

Complement fixation tests for adenoviruses gave titres below / and in neutralization tests for Coxsackie titres of /ₘ or higher were found. No IgA was detected in the nasal secretions, but in the urine there were some traces (below 10 μg/ml).

Table III. *Some gastrointestinal characteristics of the four patients lacking IgA*

		Intestinal biopsy (U/g protein)					Tolerance curve		
Case no.	Histology	Maltase (range 111–420)	Isomaltase (range 25–183)	Saccharase (range 26–138)	Trehalase	Lactase (range 9–98)	Glucose	Maltose	Lactose
1	Oedema of villi, leukocytic infiltration, lymphangiectasia	197	64	70	18	27	Normal	Normal	Normal
2	Atrophy of villi, leukocytic infiltration	54	24	28	8	10	Normal	Normal	Flat
3	Normal	—	—	—	—	—	Normal	Flat	Flat
4	—	—	—	—	—	—	Normal	Normal	Flat

Gastrointestinal tract. Gastric and intestinal roentgenograms were normal. Sigmoidoscopy showed reddened, injected mucosa. No haemorrhages or ulceration. A biopsy taken from the jejunum was normal histologically. On oral loading with maltose and, especially lactose, there was considerable diarrhoeal reaction, and the curves for these tests were pathologically flat. On oral loading with xylose 20% of the given dose was excreted during the first hours. The gastric juice was acid and culture from the faeces showed growth of Candida albicans. No blood was found in the faeces. The excretion of faecal fat was abnormally high.

Respiratory tract. Roentgenograms of the lungs were normal. Culture of the sputum showed growth of Haemophilus influenzae sensitive to penicillin but resistant to sulphonamides. Culture from the pharynx showed an ordinary growth of bacteria.

Course. No effect was obtained from treatment with ordinary gammaglobulin or gammaglobulin containing considerable quantities of IgA (Poviet). Plasma infusions were administered parenterally but again had no effect on the intestinal symptoms, and gluten-free and milk-free diet produced no improvement.

Case 4

A 39-year-old woman, gravida 1 and Para 0, uniovular twin of case 3. She had had the usual childhood diseases and had not been particularly susceptible to infections. In her youth, however she had salpingitis on three occasions. Since her early youth she had also been troubled by periodic diarrhoea, especially in the autumn and spring. During the last 18 months she had had repeated loose stools daily. No blood noticed in the stools. No loss of weight.

Physical examination. An ordinarily built woman. B P 125/90. Auscultation of heart and lungs revealed nothing abnormal. Abdominal and rectal palpation similarly showed normal conditions.

Laboratory data. Hb 1..5%. WBC and differential white blood cell count normal. ESR 34 mm/h. Plasma electrolytes, creatinine, bilirubin, alkaline phosphatases and transaminases in serum were all within normal limits, as also were the serum concentrations of vitamin B_{12} and folic acid.

The concentrations of the serum proteins are shown in Table II and those of the immunoglobulins in Table I.

The Mantoux reaction and Meinicke and Kline reactions were negative. The latex fixation test for rheumatoid arthritis was negative. The antistreptolysin titre in serum was below 90 U/ml and the antistaphylolysin titre 0.9 U/ml.

No IgA detected in the urine, and only traces were found in concentrated nasal secretions.

Gastrointestinal tract. Roentgenograms of the stomach and small and large intestines were normal. Sigmoidoscopy showed normal findings. Two attempts at obtaining an intestinal biopsy were unsuccessful. Oral glucose, maltose and fructose tolerance tests gave normal results. Lactose loading gave flat curve, but no diarrhoea occurred in association with any of these tolerance tests. On xylose loading 18% of the given dose was excreted in the urine during the first 5 hours. Faecal culture showed an ordinary growth of faecal bacteria. There was no growth of Shigella, Salmonella or Staphylococci. The stools contained no blood.

Respiratory tract. Roentgenograms of the lungs were normal. Sputum culture showed growth of Haemophilus influenzae and sparse growth of yellow staphylococci sensitive to penicillin and sulphonamides.

Course. This patient was considered to be in good general state of health, and as her bowel function became normal during the course of the investigation there appeared to be no indication for treatment of any kind.

RESULTS

The results of the immunoglobulin determinations in serum are given in Table I, where they are compared with the levels in healthy adults of the relevant age groups.

The mean values of IgG and IgA were normal. IgM was slightly high and IgD somewhat low. The mean value for IgE was low i.e. 11 ng ml. *Only one patient had a raised IgE level, 790 ng* ml, which was explainable by slight atopic symptoms.

Four women lacked detectable concentrations of IgA. Two of them (cases 3 and 4), on the other hand, showed increased levels of IgG and IgM, probably compensatory while the other two (cases 1 and 2) were to be regarded as hypogammaglobulinaemic.

The concentrations of the different serum proteins in the four patients lacking IgA are given in Table II. Low total protein and albumin values were found in the _ patients with hypogammaglobulinaemia, while the other _ showed fairly normal proteins except for the gammaglobulins.

The results of the gastrointestinal studies are given in Table III.

D-xylose, urinary excretion in 5 h (g)	Faecal fat (below 7 g)	Faecal culture	Small intestine	Large intestine
6.5	—	Normal	Normal	Normal
4.3	Normal	S aureus	Normal	Colitis-Like changes
1.3	10.8	C albicans	Normal	Normal
4 5	—	Normal	Normal	Normal

DISCUSSION

Raised serum levels of IgE occur with great frequency (30 to 80%) in atopic diseases and parasitic infections (12) High IgE concentrations in a disease would indicate the involvement of an allergic mechanism. IgE estimation can thus be used as a screening test for immediate type hypersensitivity

In intestinal capillariasis, a parasitic disease characterized by profuse, often fatal diarrhoea (21) very high IgE levels have been found (20) This might indicate that there is a relation between IgE and gastrointestinal dysfunction, especially since a considerable local production of IgE seems to take place in the mucosal membrane of several organs. However studies of coeliac disease have shown normal or low serum IgE concentrations, indicating that IgE is not involved in the immunological processes of this disease (10) Similar results were found in a preliminary study of ulcerative colitis (14)

The IgE levels found in the patients with gastrointestinal dysfunction reported here were low or normal. Thus it is highly likely that antibodies of the IgE class were not involved in this disorder at least not to a significant degree. The tendency to low levels can probably be explained by a loss of protein through the gastrointestinal tract.

Diarrhoea and sprue-like symptoms are not uncommon in acquired hypogammaglobulinaemia, and Gitlin et al. (7) noted a frequency of about 20%

Bull and Tomasi (3) have put forward several suggestions and hypotheses which might explain the pathophysiology in antibody deficiency with intestinal disorders.

McCarthy et al. (19) collected from the literature 21 cases of steatorrhoea in idiopathic hypogammaglobulinaemia. Our case 2 corresponds to this group. Some of the cases described have improved after antibiotic therapy and therefore a change of the intestinal microflora with infection by pathogenic enteric bacteria as a result has been considered to play a role (17) Our case 2 also showed a pathological faecal culture, as well as case 3 The intestinal mucosa of such patients may be normal or show atrophy of the villi with infiltration of lymphocytes, as in case 2. Treatment with a gluten-free diet has given varying results.

Hermans et al (8) described several cases of dysgammaglobulinaemia characterized by absence of IgA in the serum and normal or slightly decreased IgG These patients showed a tendency to recurrent upper respiratory infections, diarrhoea, moderate steatorrhoea and, frequently intestinal infestation with *Giardia lamblia*. *Roentgenography* of the small intestine revealed pronounced lymphoid hyperplasia, and in the intestinal mucosa normal villi, a decreased number of plasma cells and extensive lymphocytic infiltration were found.

The interest in IgA and its possible role in gastrointestinal dysfunction has increased further since several reports have been made on malabsorption in isolated IgA deficiency (4 5) Isolated deficiency of IgA is found in normal persons in a frequency of about 0.-% (1 16). Hobbs (9) investigated 11 000 hospital patients and found isolated IgA deficiency in 24 of them only one was free from symptoms. One of the patients had recurrent diarrhoea and - had malabsorption syndromes with a flat jejunal biopsy both the latter responded well to treatment with a gluten-free diet.

Crabbé and Heremans (4) reported three patients with malabsorption resembling steatorrhoea. Two of these had isolated IgA deficiency and histochemical studies of the intestinal mucosa showed a greatly reduced number of IgA-containing plasma cells but an increased number of cells containing IgM A further study (5) of one of these patients also revealed a total lack of IgA in exocrine secretions. In one of the patients the intestinal function became normal after the introduction of a gluten-free diet but again deteriorated on return to a diet containing gluten. The authors considered that the absence of IgA in the serum and exocrine secretions was not secondary to the intestinal disturbance, since the IgA deficiency remained unchanged after normalization of the intestinal function. A chance association was considered highly improbable in view of the rarity of both conditions. The authors stressed the possibility that the deficiency of IgA was the primary event and that the entire pathological condition was caused by some unknown abnormality of the immunological system. Our case 3 resembles the case described above, but it is very interesting to note the good general condition of her twin sister who also had isolated deficiency of IgA.

Binder and Reynolds (2) obtained normalization of the intestinal function in a severe case of diarrhoea with IgA deficiency and hypogammaglobulinaemia secondary to lymphatic leukaemia by treatment with fresh plasma infusions, where previous treatment with commercial gammaglobulin containing no IgA had had no effect. The authors considered that the improvement was possibly due either to the ability of the fresh plasma infusions to increase the IgG levels to a greater extent than intramuscular administration of gammaglobulin, or to an increase of the IgM IgA or other unknown factors.

Our case 2 responded very well to intramuscular administration of ordinary gammaglobulin containing no IgA. Case 3 with isolated IgA deficiency has been difficult case. No effect was obtained by the administration of ordinary gammaglobulin or Poviet gammaglobulin containing small amounts of IgA. Nor was there any effect of repeated plasma infusions on the diarrhoea. A milk free and gluten-free diet appears to have had no definite result.

It is thus not at all clear why some persons with IgA deficiency are quite healthy while others show a spectrum of diseases, including malabsorption. There are certainly several as yet unknown factors of importance for the pathogenesis of different IgA abnormalities and their relationship with different immune syndromes including malabsorption. It is doubtful, therefore, whether at this stage IgA deficiency combined with steatorrhoea can be set apart as a distinct disorder separate from other malabsorption diseases, the causes of which, in turn, are as yet not at all clear

ACKNOWLEDGEMENT

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PAIN IN ACUTE MYOCARDIAL INFARCTION

A Study of 137 Patients in a Coronary Care Unit

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Abstract. With the introduction of the coronary care units (CCU) the question of an early diagnosis of acute myocardial infarction (AMI) has become of great importance. The main criterion for admission to these units has been central chest pain. In this study the localization and radiation of chest pain has been analysed. No typical localization of the chest pain has been found in the patients with AMI compared to those admitted to the CCU but found not to have AMI. The radiation of pain to the neck and arms is, however, highly significantly more common in patients with AMI than in the controls.

The treatment of acute myocardial infarction (AMI) has undergone considerable changes during recent years owing to the introduction of specialized coronary care units (CCU). The beds in these units are considerably more expensive than beds in ordinary wards due to an extensive staff of doctors, well-trained nurses and costly equipment, and therefore an optimal use of these beds is essential. Our diagnostic rate during 1968–1969 was, however, only 41% (12). This prompts a better differentiation of patients for CCU care. In the present study an investigation has been made of the most important admission criteria used, i.e. that of central chest pain. Central chest pain has been defined as pain within the medioclavicular lines. The purpose was to investigate whether there is any possible difference concerning the localization of the chest pain in those patients who subsequently received the diagnosis of an AMI as compared to those who did not. Furthermore, an analysis has been made with regard to the frequency of radiation of the pain to the arms and the neck.

MATERIAL AND METHODS

The general therapeutic methods used in the unit have been previously described (2). The guiding policy for admitting patients to this CCU is the fulfilment of at least one of the following criteria. 1. Central chest pain lasting for more than 15 min within the last 48 h. 2. Frank pulmonary edema without previously known valvular lesion. 3. Shock without suspicion of acute hypovolemia or intoxication. 4. Syncope with electrocardiographic evidence of AMI. 5. *Intractable* angina pectoris. The criteria for the diagnosis of AMI in these patients has been fulfilment of *a*, *b* or *c*. (*a*) Appearance of pathological Q waves and or appearance or disappearance of localized ST elevation followed by T inversion in two or more of twelve leads. (*b*) The GOT values (serum aspartate aminotransferase) of 40 U or more and with maximum about 24 h after onset of symptoms in combination with lower GPT values (serum alanine aminotransferase) with maximum after about 36 h and or two HBD values (α-hydroxybutyrate dehydrogenase) exceeding 75% of corresponding LDH values (lactic dehydrogenase) higher than 400 U, with maximum about 60 h after the onset of symptoms, or a combination of one GOT-GPT value and one HBD-LDH value elevated as stated above. (*c*) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms.

In this study 137 patients, 86 men and 51 women, were investigated between Nov. 1 1969 and Jan. 31 1970. Of these 51 patients subsequently received a diagnosis of AMI, 34 men (aged 43–81, mean 61 years) and 17 women (aged 58–79 mean 68 years). Five patients, three men and two women, received a diagnosis of "suspected infarction" and in 81 patients, 49 men (aged 41–87 mean 59 years) and 32 women (aged 49–80, mean 64 years), the diagnosis of AMI was excluded. These patients will subsequently be referred to as controls. Patients with suspected infarction are not included in this study.

The author took the history of each patient as regards the pain and the radiation of pain. The patients were asked to demonstrate the area of pain over the chest with their fingers and to indicate the presence of radiation

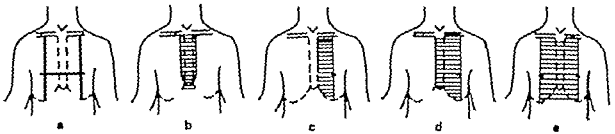


Fig 1 (*a*) Mamillary plane, medioclavicular lines. (*b*) Retrosternal area. ( ) Left parasternal area. (*d*) Left retroparasternal area. (*e*) Para-retroparasternal area.

to the neck or left and/or right arm. The results were directly registered by plotting the area of pain on to simple drawing of the chest, the neck and the arms (Fig. 1).

The chest was divided into different sectors. Analysis of pain was made with regard to site above or below the mamillary plane, retrosternally only parasternally only as well as retro-parasternally

RESULTS

Pain

No difference was found regarding the site of the pain in patients with AMI as compared to the controls. 22% of the patients with AMI indicated pain above the mamillary plane as compared to 21% of the controls. Below the mamillary plane the rate was 27% against 20%. Pain covering the mamillary plane was indicated in 51% by the patients with myocardial infarction against 59% by the others. Pain localized only retrosternally was indicated by 12% of the patients with myocardial infarction against 16% of the controls. Pain strictly outside the retrosternal area was indicated by 35% in both patient groups. Pain covering the area from the sternum to the left medioclavicular line was indicated by 6% of the patients with infarction against 5% of the controls. Chest pain from the right to the left medioclavicular line was indicated by 39% of the patients with AMI against 36% by the controls. None of these differences are significant.

Radiation

Seventy-one per cent of the patients with AMI indicated radiation of pain to the neck as well as to the left and/or right arm as compared to 39% of the patients without AMI This difference is highly significant ($p < 0.001$). Radiation only to the neck was indicated in 7% in both groups. Radiation of pain to the left arm alone was indicated in 29% against 20% ($p < 0.05$). Radiation to the right arm was indicated by only one patient. Radiation of pain to both left and right arm was present in 33% of the patients with AMI as compared to 12% of the controls ($p <$

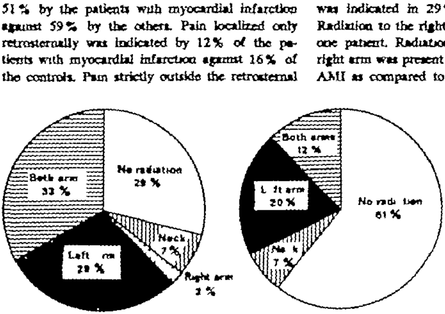


Fig. 2 Radiation of pain. (*a*) Patients with AMI. (*b*) Controls.

0 01). Radiation to the neck in combination with radiation to one or both arms was indicated by 8 % and 6 % respectively of the two groups (Fig.).

No chest sector was significantly over-represented regarding radiation to the neck or either arm.

Other symptoms

Ten patients indicated diffuse pain in the whole chest of whom four had myocardial infarction and six were controls.

Three patients had no pain One patient in each group was admitted to the CCU with frank pulmonary edema as the only symptom. The third came in with syncope and ECG evidence of AMI.

Nine patients indicated pain or discomfort in the epigastrium in connection with chest pain, three with AMI and six without this diagnosis.

as well as over or beside the retrosternal area has been analysed. With reference to the common opinion that retrosternal pain is the rule in patients with cardiac pain, it was interesting to note that 35 % of the patients in both groups placed all their pain outside the retrosternal area. The radiation to the neck or arms is, however highly significantly more common in patients with AMI than in the controls ($p < 0.001$). Radiation to the right and left arm at the same time was indicated by 33 % of the patients with AMI as against only 12 % of the other patients ($p < 0.01$)

In this study no attempt has been made to estimate the intensity quality and duration of the pain, but these characteristics as well as other clinical features ought to be evaluated for a better diagnostic prediction on admission to hospital of patients with a suspicion of an acute myocardial infarction.

DISCUSSION

With the introduction of the CCU the question of an early diagnosis of the AMI has become of great importance. The main criterion for admission to these units has been central chest pain (2)

According to the literature the coronary pain is often indicated as central, substernal or mid sternal without any more detailed analysis concerning the distribution. Rosenbaum and Levine (9) stated that the distribution of pain was of little value in predicting the location of the infarction. The frequency of radiation is a subject of controversy Bean (1) found radiation in only 50 % of 144 patients. Sievers, in his study (11) found it in 86 % of more than 1 200 patients. Concerning the radiation to the left or the right arm Wyburn-Mason (15) said that radiation to the left arm alone occurs about 25 times more often than to the right arm, radiation to both arms being about one-fourth as common as that to the left arm alone.

In this study the criterion of central chest pain and its radiation to the neck and arms has been analysed.

No typical localisation of the chest pain has been found in the patients with AMI compared to those admitted to the CCU but subsequently found not to have a myocardial infarction The distribution above and below the mamillary plane

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INTRAVENOUS ADMINISTRATION OF ESSENTIAL AMINO ACIDS TO UREMIC PATIENTS ON LOW PROTEIN DIET

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Abstract Patients with chronic renal disease and severe uremia (serum creatinine above 10 mg/100 ml) were treated in 1965–67 with a restricted protein diet with addition of essential amino acids in tablet form, as described by Giovannetti and Maggiore. There were however often practical difficulties in carrying out an oral amino acid substitution. Parenteral administration of eight essential amino acids was introduced in 1968–70 for 19 severely uremic patients once to twice a week for 1–14 months. The patients preferred parenteral administration of the essential amino acids, and few side-effects were observed. Amino acid analysis of plasma showed all amino acid concentrations in the normal range in four severely uremic patients, who received a low protein diet (about 15 g/day) and 50 g essential amino acid/week. No signs of deficient protein synthesis were found. It is extremely difficult to calculate the optimum amino acid supply required by uremic patients. The fact that one of our patients had a life extension of 14 months indicates that the amino acid balance was reasonably adequate. In patients with a quickly developing renal deterioration the advantage of strict protein rationing is minimal. In the very slowly developing diseases there may be a gain of several months.

Patients with chronic renal disease are usually prescribed a low-protein diet in order to lessen the accumulation of nitrogen metabolites. It has long been known that urea, one of the end products of protein metabolism, can be used to a certain extent for a new synthesis of non-essential amino acids. This was first tried out in practice by Giordano (3) and Giovanetti and Maggiore (4). According to the two latter, the patient was kept on a basic protein diet equal to 1.0–1.5 g nitrogen and eight essential amino acids as recommended by Rose et al. (10, 11). Patients on this diet proved to have less urea-N in serum and lived longer than uremic patients on an ordinary diet. This principle has been used in Sweden, where essential amino acids have been given in tablet form, Kidnamin®. Quite recently Richards et al. (9) have studied the incorporation of ^{15}N-ammonia with a low-protein diet in healthy persons and uremic patients. In the former group there was an increased incorporation of ^{15}N the lower the diet was in proteins. The uremic patients had an incorporation of 700–975 mg amino-N every 24 h (9). The daily requirement of eight essential amino acids for adults has been estimated by Rose et al. (10) to be a minimum of 6.35 g/day (Table I). The requirement necessary for uremic patients is not quite certain. Like most Renal Departments we give the essential amino acids in tablet form, Kidnamin®. There were, however, not many uremic patients, suffering as they do from nausea, who could manage to take fifteen large unpleasant-tasting tablets every day. We have, therefore, tried giving the essential amino acids intravenously once a week. Our intention has been to analyse eventual side-effects and to see whether there was any sign of a diminished concentration of essential amino acids.

MATERIAL AND METHODS

The material consisted of 14 men and 5 women aged 34–73 years. All had severe uremia, and in the beginning a creatinine concentration of about 10 mg/100 ml serum or above. Amino acid solution (Vitrum, Stockholm) was administered in doses of 1 000 ml for three hours, or 500 ml/day two days a week for 2–60 weeks. If one leaves out of account the excretion of amino acids via the kidneys, then the patients have been administered 7.14 g essential amino acids a day or 10% above the minimum requirement of 6.35 g/day. A more or less vegetarian diet was given as well, with an estimated protein con-

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Table I. *The daily requirement of eight essential amino acids for adults estimated by Rose*

Amino acid	Daily need, minimum (g)	Content of Vitrum amino acid solution no 6467 (g/100 ml solution)
L-isoleucine	0.70	0.55
L-leucine	1 10	0.87
-lysine	0 80	0.63
L-methionine	1 10	0 87
-phenylalanine	1 10	0.87
-threonine	0.50	0.39
L-tryptophan	0.25	0 20
-valine	0 80	0.63
	6.35	5 01

tent of about 15 g/day Amino acid concentration in an ultrafiltrate of plasma was analysed in Beckman 120 C amino acid analyser after deproteinizing in Diaflocell (6). A new lithium citrate buffer system was used which separates more amino acids than previously (1). 2-amino-3-guanido-propionic acid was used as an internal standard. The chromatograms were evaluated by an integrator Infotronics CRS 100 A. Albumin was determined by radial immuno-diffusion with Partigen plates (Behringwerke).

CASE REPORTS

Case 1

The patient had chronic pyelonephritis. Uremia since 1963, small kidneys. She was admitted to the hospital f tiredness and vomiting. She had urea-N 234 and creatinine 9 7 mg 100 ml serum. On Nov 17 1968, her diet was restricted to ery low protein content, consisting of 10–15 g protein every 4 h. Each week she was then given intravenously first 500 ml, and then after some weeks 1 000 ml of 5% amino acid solution with essential amino acids each week. The patient' condition improved considerably. Her uremia decreased, serum creatinine went down to 6.0 mg 100 ml at the lowest, urea-N was down to 50 mg 100 ml at the lowest. She then came once or t ice week to Umeå Hospital for amino acid administration. She continued with her low-protein diet the whole time. She was given concentrated blood, about 200 ml/month. She felt well for about a year was up and managed light housework, but was tired. She had occasional diarrhea. She had slight nausea when given 1 000 ml amino acids but hardly any when given 500 ml. Her renal function continued to worsen. She had on Dec. 4, 1968, 50 mg 100 ml urea-N in serum; March 5 1969 96 mg; June 4, 1969 136 mg; Sept. 10, 1969 160 mg; and Jan. 16, 1970, 300 mg 100 ml. Her weight increased from 51 kg on Nov 4, 1968, to 56 kg Dec. 4 1968, 62.3 kg Sept. 4, 1969 and 60 kg Jan. 1., 1970. No positive edema. Total protein on Nov 4, 1968, 6.6 g. Sept. 10, 1969 7.0 g 100 ml. Hand strength was low about 250 mmHg; this was constant. She was at home for nine out of fourteen months. She died on Jan. 16, 1970, after three weeks unconsciousness. During these three weeks amino acids but no protein were administered. The last analysis of her amino acid concentration in serum was performed 2 days before death. Albumin concentration in serum was on Dec. 11 1968 3.6, Jan. 15 1969 4.0, Feb. 12, 1969 3.9 and March 26, 1969 3.7 g/100 ml.

Case 2

The patient has double-sided cystic kidneys. He has an increasing urine poisoning. On Nov 8, 1968, the serum creatinine concentration was 17.2 mg/100 ml, urea-N 130 mg/100 ml serum. He had nausea with omiting and headache. On Nov 8, 1968 intravenous administration of essential amino acids was started. He received 500 ml of 5% solution in the first month; after Dec. 11, 1968 1 000 ml once a week. H stood the infusions well. Urea-N fell to 77 mg/100 ml serum at the lowest, and creatinine concentration to 1..4 mg/100 ml serum. The patient's nausea, omiting and headache improved considerably. He complained, however, of itching, numbness and muscular weakness f the hands, but he had had these complaints before the amino acid infusion as begun. Hand strength fell from 575–525 mmHg on the dynamometer. As w were afraid that the patient would eventually develop polyneuritis, hemodialysis was started on Jan. 28, 1969 Serum creatinine was then 13 4 mg, and urea-N 80 mg/100 ml serum. The patient is still on hemodialysis and feels well. During amino acid treat ment, the protein content in serum increased from 6.6 to 8.0 g/100 ml. Weight fell from 80 kg to 75 kg, possibly due to disappearance of edema.

Case 3

The patient had chronic pyelonephritis with small kidneys Uremia as diagnosed in 1966. Creatinine concentration increased to 11 mg/100 ml serum, and urea-N to 77 mg/100 ml serum on Nov. 4, 1968. He was increasingly tired, had continuous nausea, omiting and breathlessness. On Nov 5 1968, low protein diet was started with protein content of 15 g every 24 h. In addition, first 500 ml, and after some weeks 1 000 ml, 5 amino acid solution of essential amino acids ere given intravenously each week. The patient's nausea improved, but he was still very tired. Serum creatinine fell at the lowest to 9.4 mg 100 ml, and urea-N to 74 mg/100 ml serum. The patient's diuresis, however decreased successively from 1 700 ml to about 600 ml/24 h. On Feb 4, 1969 creatinine concentration was 9.8 mg 100 ml serum, urea-N 102 mg/100 ml serum. Total protein on Nov 4 1968 was 7.1 g, and on Feb. 4, 1969 6.9 g/100 ml. Body weight fell from 60 kg on Nov 4 1968, to 57 kg on Feb. 4 1969 The patient was tired and eak the whole time On Feb. 15 1969 he developed high fever and lung edema and died.

Case 4

The patient had chronic pyelonephritis. Creatinine as 6 mg in 1967 On Nov 11 1968 the creatinine as 1..4 mg, urea-N 118 mg/100 ml serum. H general health, however was quite good. A low protein diet was started, 15 g every 4 h, and infusion of essential amino

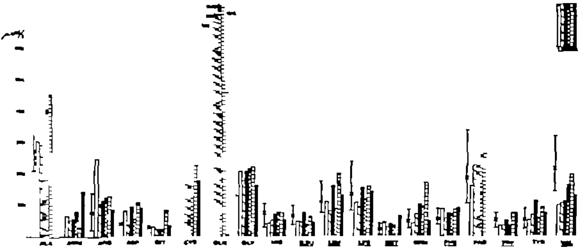


Fig. 1. Case 1. Amino acid concentration in plasma. Column I before treatment with essential amino acids, II after 2 months, III after 3, IV after 5, V after 9, VI after 14 months.

acids was given intravenously, first 500 ml and then, after some weeks, 1 000 ml [illegible] week of 5% amino acid solution. Kidney function, however, continued to worsen rapidly. Urine output diminished. On Jan. 14, 1969 the creatinine concentration was 4.8 mg. and urea-N 219 mg/100 ml serum. The patient was becoming increasingly weaker, had nausea, vomiting and breathlessness. Blood pressure 200/110. We were forced, therefore, to start hemodialysis on Jan. 15, 1969. On Nov. 11, 1968 the patient's protein in serum was 6.8 mg., on Jan. 14, 1969 6.1 mg. Weight remained constant during amino acid treatment. Eventually kidney transplantation was performed (donor identical twin). He is in extremely good health.

RESULTS

Occasionally the patients felt slight nausea during infusion of 1 000 ml amino acid solution. If the infusion was divided into 500 ml each time, this feeling was considerably reduced. There has been very little vomiting, and one or two cases of slight dizziness. The frequency of nose-bleeds and bruises has probably increased slightly, but as this is very usual with uremic patients it is difficult to judge whether it is due to the administration of amino acids. There were no serious he-

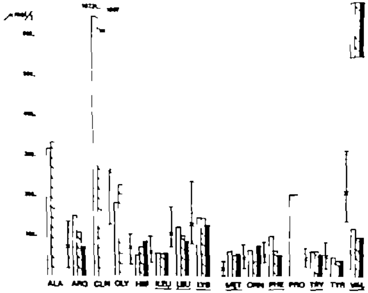


Fig. [illegible]. Case [illegible]. Amino acid concentration in plasma. Column I before treatment with essential amino acids, II after one, and III after two months' treatment.

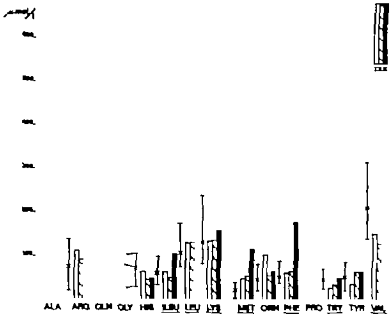


Fig 3 Case 3. Amino acid concentration in plasma. Column I before treatment with essential amino acids, II after one, and III after two months treatment.

morrhages, and no other side-effects were noted.

The concentration of 15–18 individual amino acids was determined in four uremic patients. All had severe uremia with creatinine above 10 mg/100 ml and initially a normal concentration of amino acids in serum. The analyses have since been repeated once a month for 2 months in three of the patients, and once a month for 5 months and then every other month up to 14 months in one patient (case 1). Of the original 19 patients who received amino acid treatment, nine were transferred to hemodialysis inside two months, five died from their basic disease (myeloma with uremia, pyonephritis with cardiosclerosis and severe cardiomegalia, pancreas neoplasma with uremia, senility with ureteral strictures, and uretostomia and infection with severe uremia) within two months. In one case the analysis was unsuccessful and thus four patients remained longer than two months. To analyse large series

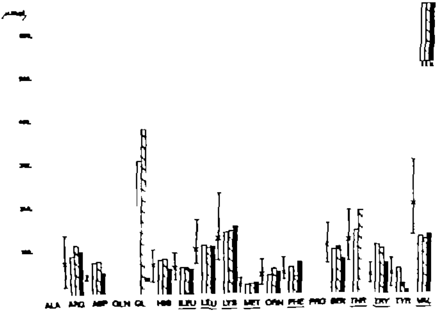


Fig. 4 Case 4 Amino acid concentration in plasma Column I before treatment with essential amino acids, II after one, and III after two months treatment.

would incur unreasonable costs. Thus four patients have been kept under observation for at least three months.

Amino acid analyses

The concentration of essential and nonessential amino acids did not diminish in any of the patients under our care (Figs. 1–4). Enough amino acids were administered to the patients to prevent a lowering of the serum concentration of amino acids during these 2–14 months.

DISCUSSION

The greatest difficulty has been to determine how much protein the patients get through their diet. They have not been treated in a metabolical department. For the most part they have been in their homes. From what we can gather from the patients, relatives or nursing-staff, there is no reason to suppose that the patients have eaten more protein than prescribed. They have not taken meat, fish, cheese, eggs or milk. In spite of this, as there is usually so much protein in Swedish food, their protein intake is estimated at about 15 g daily. On the other hand, many uremic patients have poor appetites, a distaste for certain foods and vomiting attacks.

The patients have been aware that protein was very bad for them and might cost them their lives. Nevertheless we have not been able to reach the low level of protein intake, 6–10 g a day as prescribed by Giordano (3), and Giovanetti and Maggiore (4). With starvation there is a rapid increase of plasma concentrations, valine, leucine, isoleucine, methionine and alpha-butyric acid. Of the remaining amino acids, alanine is the one that decreases most (2). With severe protein deficit (kwashiorkor), without starvation, there is a low plasma concentration of valine, leucine, isoleucine and threonine. The concentration of alanine may increase in the case of a slight deficit of protein, but decreases in the case of severe deficit (5). Our analyses of amino acids show that our patients are getting sufficient amino acids. Further criteria for protein insufficiency such as a reduction of albumin or total serum protein, body weight and hand strength, are difficult to interpret in uremic patients. The basic disease is not cured by the diminished protein intake, but merely progresses, as does ultimately the uremia. We could find no definite signs of protein insufficiency.

Additional histidine is needed for a normal protein synthesis in children and young adults (7). Giovanetti and Maggiore (4) did not use any additional histidine, and we have not seen any signs of histidine insufficiency in our patients.

It is uncertain whether uremic patients need less amino acids than healthy patients. They lose less perhaps, through urine excretion and probably have a smaller protein synthesis owing to the sedentary life they lead.

It is extremely difficult to say anything about the optimum amino acid supply required by uremic patients. On the one hand every mg nitrogen increases their uremia, on the other hand they should receive a minimum supply so as not to increase the protein catabolism above normal. Amino acid analyses show the concentration to be, for the most part, within normal limits. The fact that one of our patients had a life extension of 14 months indicates that the amino acid balance was reasonably adequate. The indication for a low nitrogen ingestion in uremic patients is to lengthen the time they can exist with very little renal function left. The important advantage is for those patients who have reached a serum creatinine concentration of 10 mg/100 ml or higher. In patients with a quickly developing renal deterioration the advantage of strict protein rationing is minimal. In the very slowly developing renal diseases there may be a gain of several months. It is uncertain, however, what can be won by giving strict low-protein diet (15 g day or less) with the addition of intravenous amino acids, compared with low-protein diet with a protein content of 25–40 g day without addition of amino acids.

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SERIAL OBSERVATIONS ON LEFT VENTRICULAR DYSFUNCTION IN ACUTE MYOCARDIAL INFARCTION

I Gallop Sounds Ventricular Asynergy and Radiological Signs

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Abstract The occurrence and significance of various signs of left heart pump failure has been studied serially in 50 patients with acute myocardial infarction (AMI) 1 2, 3, 5 10, 15, and 20 days after the onset of symptoms. The clinical severity of AMI, the findings on cardiac palpation and auscultation, the electro- phono- and kinetocardiographic findings and the radiological signs of left ventricular failure were evaluated. Evidence of left ventricular dysfunction was observed in all patients: atrial gallop in 100% (audible in 82), high wave kinetocardiographically in 92% ventricular gallop in 90% (audible in 80%), papillary muscle dysfunction in 58% ventricular asynergy kinetocardiographically in 100% (palpable paradoxical pulsation in 86%), pulmonary rales in 70% pulmonary vascular congestion in serial X-rays in 98% and enlargement of the heart in 42% These signs developed during the first days, also in patients with normal-sized hearts, and persisted in the majority Because of their high prevalence, auscultatory and phonocardiographic signs of myocardial dysfunction and, similarly ventricular asynergy as demonstrated by palpation and kinetocardiography if assessed cross-sectionally are not useful in assessing the hemodynamic severity of AMI or its prognosis. However serial studies indicated that changes in the clinical course of AMI are accompanied by parallel changes in the clinical signs of left ventricular dysfunction. Radiological signs of pulmonary vascular congestion and electrocardiographic signs of left atrial overloading are valuable in the assessment of the clinical severity and course of AMI.

Since the institution of coronary care units, the monitoring and treatment of arrhythmias has resulted in a decrease of hospital deaths in cases of acute myocardial infarction (26, 34). Hemodynamic deterioration due to left ventricular pump failure is now becoming the leading cause of infarction deaths in these units. However the basic mechanisms of pump failure in myocardial infarction and its hemodynamic consequences are only recently becoming understood.

In the present study we report serial clinical, phono- and kinetocardiographic and radiological observations on the prevalence and value of signs of left ventricular dysfunction in patients with acute myocardial infarction of varying severity

MATERIAL

Fifty patients with AMI, consecutively admitted within 4 h from the onset of symptoms to the Intensive Care Unit of this hospital, were studied serially Thirty-six patients were admitted within 6 h after symptoms. The criteria for AMI were: history of chest pain, pattern of acute infarction in serial ECGs, and rise of serum GOT and LDH levels. There were 9 females and 41 males, the age range being from 32 to 80 years (mean 5 9 years) Previous history revealed exertional angina pectoris of duration of more than one month before infarction in 18 patients, previous myocardial infarction in 10 patients, and arterial hypertension in 10 patients. None had congenital or other acquired heart disease

Basal pulmonary rales, showing shift to dependent parts on change of position, were observed in 35 patients (on admission in 23, at discharge in 14); severe clinical pulmonary edema occurred in 6 of these patients Cardiogenic shock (peripheral vasoconstriction, metabolic acidosis, oliguria, cerebral dysfunction, together with systolic arterial pressure below 90 mmHg during at least 30 min) developed in 8 patients Six patients were resuscitated and 6 needed assisted ventilation. Digitalis was administered to 39 patients and isoprenaline or pressor agents to 4

The patients were divided into three groups according to the clinical severity of AMI (60).

Group I Uncomplicated infarctions although showing occasional extrasystoles or other transient insignificant arrhythmias, no clinical evidence of heart failure (15 patients)

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Table I. *Electrocardiographic assessment of infarction in 50 patients*

Transmural infarction	46
Nontransmural infarction	4
Left bundle branch block	2
Right bundle branch block	1
Location*	
Anteroseptal	0
Anteroseptal + anterior	11
Anterior	3
Anterolateral	4
Extensive anterior	7
Total anterior	25
Inferior	9
Inferolateral	6
Inferoposterior	3
Inferoposterolateral	4
Posterolateral	2
Posterior	1
Total posterior	25

* ref. (19).

Group 2: Moderate infarctions not belonging to group 1 or 3 (23 patients).

Group 3: Severe infarctions complicated by frank pulmonary edema or serious cardiovascular failure, cardiogenic shock, or progressing to death (12 patients).

Nine patients died in the hospital.

METHODS

The patients were examined clinically by the same observer during their 1st, 2nd, 3rd, 5th, 10th, 15th and 20th day in hospital. Auscultatory and palpatory findings were recorded first and, in order to evaluate the sensitivity of the clinical and graphic methods, phonocardiograms (PCG) and kinetocardiograms (KCG) were recorded thereafter.

Auscultation

Selective auscultation and interpretation of heart sounds and murmurs were performed using both the bell and the diaphragm end-piece of the stethoscope. In addition to the supine position, all patients were auscultated in the left lateral position, in order to recognize the low pitched atrial and ventricular gallop sounds, and the mostly faint mitral systolic murmurs of acute papillary muscle dysfunction (19).

Phonocardiography

Apical PCGs were recorded in both quiet respiration and expiratory apnea with an Elema-Schönander EMT 25 microphone and a direct-writing ink-jet apparatus (Mingograph 81 Elema-Schönander) at a paper speed of 50 mm/sec. High-pass filters with nominal frequencies of 35, 70, 140 and 250 cps were used to record both gallops and high frequency murmurs. In the interpretation of the PCGs special care was paid to the exclusion of artifacts (19). Atrial gallop was identified as low frequency vibrations coinciding with the wave of the KCG and occurring between the inscription of the P wave and the beginning of QRS, with its first 30 msec included, in the ECG. As regards the atrial gallop, the atrial component type was not separately noted. Ventricular gallop was identified as low frequency sound 0.10 sec or later after A_2, synchronous with the peak of the rapid filling wave of the KCG. For rough phonocardiographic quantitation the mitral regurgitant murmurs noted were graded into two groups as described previously (21).

Palpation

Precordial palpation was performed to determine the character of the apical beat and the occurrence of palpable paradoxical cardiac pulsation, its localization, shape and degree, and changes in these items in the course of time.

The apical impulse was considered normal if located in the 5th intercostal space in the midclavicular line or medial to it, and if felt as brief early systolic thrust (25, 47). Paradoxical cardiac pulsation was sought for in held expiratory pause by palpating the precordium with the palm of the hand, using moderate pressure. Paradoxical pulsation was noted as present if: 1) systolic thrust distinctively separate from the apical impulse was felt; 2) heaving apical impulse was included if reaching up to the 3rd intercostal space or to the epigastric area, to distinguish it from the heave of pure left ventricular hypertrophy; and 3) if marked variation in the degree of apical pansystolic pulsations of smaller area was found on serial palpation, related to the stage of AMI.

Duration of the systolic palpable thrust was denoted as "*pansystolic bulge*" if it reached the 2nd heart sound in simultaneous auscultation, or as *abnormal pulsation* if its duration was less, and was labelled as early, mid, and late systolic thrusts. The following grading of the paradoxical cardiac pulsation was used: 1) absent, 2) present only at expiratory phase, 3) present throughout the normal respiratory cycle, 4) marked, and 5) visible.

Kinetocardiography

Equipment and procedure. Precordial movements were serially recorded by the KCG method, which records absolute displacement in relation to a fixed point outside the chest (10–12). The Elema-Schönander EMT 439A transducer was attached to a specially constructed movable stand, fixed to a platform of the Mingograph 81 six-channel recorder. The important characteristics of the recording system are: faithful recording of the absolute precordial movement, flat frequency-amplitude response of 0.1 to 200 cps, and a time constant of 1.5 sec. The sensing probe could be conveniently and accurately directed and lightly pressed perpendicular to selected points on the chest, the heavy apparatus and the locked rigid stand giving a firm support to the pick-up.

Recordings were secured in apnea at the end of normal expiration from 9 points, K33, K43, K24, K34, K44, K54, K35, K45, KEM, and from the common carotid artery at a paper speed of 50 mm/sec. The first

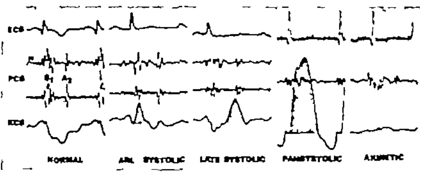


Fig. 1. Different types of KCG systolic precordial motions encountered.

numeral in the subscript of K refers to the vertical line corresponding to similarly labelled V-leads in the ECG, and the second to the intercostal space (11–12). The patients were in horizontal supine position during recording. ECG lead 2 and PCGs were recorded simultaneously on separate channels as references. The baseline of the displacement curve was selected at the point 0.04 sec after onset of electrical ventricular activation (5). For comparison of the sequential changes in given patient the amplification of the recording apparatus was always the same throughout the studies.

Definition of changes. The tracings were classified for site, area, absolute and relative amplitude, configuration and duration of the pathological systolic outward movement. The amplitude of paradoxical outward movement was measured as the distance between the summit of the systolic upward movement and the baseline. The ratio of this distance to the total amplitude was denoted as "bulge/T ratio". The type of pulsation was termed "*pansystolic bulge*" if the positive deflection crossed the baseline at early systole and had duration of 0.20 sec or more (although in 4 patients the bulge did not reach the 2nd heart sound by this definition), *abnormal pulsation* if the positive deflection had duration of 0.09 to 0.20 sec in any part of the systole, and "*normal*" if of shorter duration (5). The normal upper limit of duration of the left ventricular thrust in the KCG is 0.085 sec (including two S.D. above the mean) (5). The shape of the paradoxical pulsation was denoted as early, mid, late or pansystolic, which was further subdivided into ascending, plateau, bifid and descending patterns (Fig. 1). The percentages of both the pre-ejection retraction and the ... ard retraction below the baseline in the tracing showing the most pathological changes were measured.

The ratio of the height of the wave to the greatest total amplitude of the KCG tracing (a/T ratio) was determined both at the apex and at the site of maximum paradoxical pulsation. Ratios exceeding 20% were considered abnormal (22).

Electrocardiography

The site of AMI was assessed as anterior or postero-inferior, with the further subdivisions shown in Table I, and its extent as transmural and nontransmural. Left atrial overloading was determined by the measurement of P terminal force (19, 21) on the 1st, 5th and 20th day of infarction. The patients had sinus rhythm with the exception of one with atrial fibrillation.

Chest X-rays

The chest X-rays were taken from all patients on the 1st day. X-rays were available from 45 patients and at discharge 3 weeks later or before death from 48 patients. The first films were postero-anterior and taken supine in bed with a portable apparatus and film-to-focus distance of 1 m. The films at discharge were postero-anterior and lateral and were taken in the standing position with film-to-focus distance of m. The heart was considered enlarged if the cardiothoracic ratio was greater than 55% in the first-day films, or if the heart volume was greater than 500 ml/m² BSA in the films at discharge. Pulmonary vascular congestion due to left heart failure was evaluated independently of clinical findings and scored 0 to 3: 0 = normal, 1 = pulmonary venous dilatation only, 2 = interstitial edema, cloudiness, septal lines and the reversion of the normal vascular pattern with upper lobe vasodilatation in association with severe lower field vasoconstriction, 3 = frank alveolar pulmonary edema (19).

RESULTS

Atrial gallop

Atrial gallops were found in serial PCGs during the hospital stay in all the 49 patients with AMI who had sinus rhythm (Figs. 2 and 5). In the PCGs taken on admission atrial gallop was present in 42 patients, and persisted up to discharge in all but 5 patients. Serial auscultation missed the atrial gallop sound in a total of 18% of all the auscultation-phonocardiographic comparisons, mainly when the sound presented only as an atrial component with its fusion to the immediately following first heart sound. Thus atrial gallop was audible in 82% of the patients in sinus rhythm. Of the 9 patients who died in hospital all had auscultatory atrial gallops terminally.

Ventricular gallop

Ventricular gallop was also very commonly found at the serial PCG examinations, occurring in

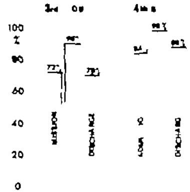


Fig 2 Prevalence of atrial and entricular gallops on admission, at discharge and totally in serial PCGs of 50 consecutive patients with AMI

total of 45 patients (90%) Its prevalence on admission was 35/50 (72%) and 29/41 (70%) at discharge (Fig. 2) Compared with atrial gallop, ventricular gallop escaped notice less often, in 10% of cases, at clinical auscultation The prevalence of audible ventricular gallops was thus 80% Six of the 9 patients who died later had on admission an audible ventricular gallop which persisted, and in 2 additional patients it appeared before death.

Quadruple rhythm

Quadruple heart rhythm due to simultaneous atrial and ventricular gallops occurred in 90% of the patients when all the recorded PCGs were reviewed, as all the patients in sinus rhythm with ventricular gallop also had atrial gallop.

Papillary muscle dysfunction

Acute development of apical murmur of mitral incompetence was noted in 29 patients (58%) by serial auscultation and confirmed by PCG The clinical criteria used in considering the murmur to be due to ischemic papillary muscle dysfunction have been given previously (19 20). In 7 patients the main type of the characteristically variable murmur was graded as insignificant (grade 1 regurgitation. early and late systolic, protomesosystolic or decrescendo pansystolic), and in 22 patients the murmur was definitely pansys-

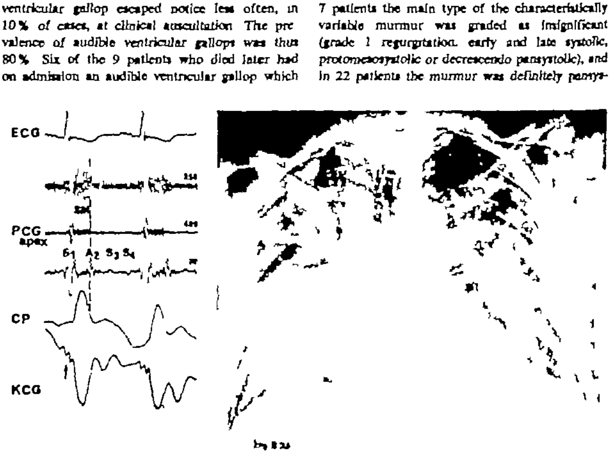


Fig 3 A 52-year-old patient with rupture of the posterior papillary muscle following AMI A harsh grade 4 pansystolic apical piadle-type murmur (SM) of severe mitral incompetence developed on the 2nd day and resulted in severe pulmonary edema, terminating fatally on the 6th day Note the short ejection time in the carotid pulse tracing (CP) and the rapid emptying of the left ventricle due to free mitral leak before the rupture distinctive paradoxical bulge was present.

Table II. *Prevalence of the main shapes of paradoxical bulge in KCG related t location of infarction*

Location of infarction	Pansystolic bulge (no. of cases)					Minor abnormality (no. of cases)				
	Ascending	Plateau	Bifid	Descending	Total	Early	Mid	Late	Total	Total
Anterior	12	4	4	0	20	2	1	2	5	25
Posterior	6	5	7	1	19	4	1	1	6	25
Total					39				11	50

tolic of types plateau, crescendo or crescendo-decrescendo (grade _ regurgitation) (21) In addition, 4 patients had already previously a pansystolic mitral regurgitant murmur

In 17 patients acute mitral incompetence re mained permanent after its appearance· it was inconstantly present in 6, and disappeared totally in 6 patients. The murmur was mainly faint and soft, grade 1–4/6, often detected only in the left lateral position and easily overlooked without care ful serial auscultation. In 3 patients who died with acute mitral incompetence the autopsy showed necrosis without rupture of the papillary muscle, involved by the free wall infarction while in 3 other patients without murmur the papillary muscles were found at autopsy to be uninvolved.

Raptu e of papillary muscle

One patient died because of rupture of the posterior papillary muscle at its base due to involvement by a large posterior infarct (Fig. 3).

Pericardial friction rub

This was noted in 8 patients (16%)

Ventricular asynergy

Prevalenc The fundamental finding in the serial KCG studies was pathological precordial systolic outward motion, which was observed in every patient at some time during the study ir respective of the electrocardiographic site of infarction (Table II)

The time of maximum paradoxical pulsation appears in Table III. 90% of the bulges were recordable already on the first day· in only 5 patients did the pathological cardiac impulse develop later The maximum amplitude of the pathological pulsation occurred early in 58 .. of the patients within the first 3 days after onset of infarction. Large pansystolic bulges were recorded in some patients already 1 h after the onset of symptoms. In 11 patients the maximum bulge did not occur until discharge.

Location and area of paradoxical movement The center of the paradoxical pulsation observed at the time of its maximum amplitude is shown in Fig. 4 The locations of the maximum thrusts in all the serial examinations showed sometimes minor variations, but they still had the same general pattern as at the time of the maximum bulge in relation to anterior or posterior infarc tion. In *anterior infarction* the pathological pulsa tion occurred mostly in the left parasternal so apical area (K34 K35 K45 K44) while *posterior infarctions* produced bulges mos com

Table III. *Time of maximum amplitude of bulge n KCG*

Day of study	1		3	5	10	20
No. of cases	12	11	6	10	5	6

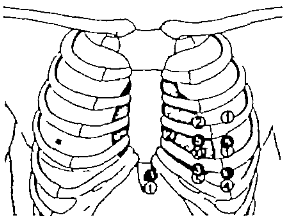


Fig. 4. Precordial distribution of maximum systolic paradoxical outward motion in anterior (open circles) and posterior (filled circles) infarctions.

Table IV *Area of bulge in KCG related to location of infarction.* (*no of cases*)

	Anterior	Posterior
1 or 2 intercostal spaces	1	11
3 or more intercostal spaces	24	14
Total	25	25

monly in the apical and adjacent areas (K45 K35 K34, K44 KEM)

The paradoxical outward movement of the heart displaced the precordium often very widely with propagation of the impulse from the point of maximum over several intercostal spaces, but there was no definite correlation to the clinical severity of the infarction. The point of maximum was located in some instances very far from the normal apical impulse, for instance in points K33 K43 K54 or in the epigastrium. Anterior infarctions showed characteristically a large total area of pulsation, which in all but one case (96 %) was felt or recorded in at least three interspaces (Table IV) This occurred regularly also in radiologically normal-sized hearts (Fig. 5). Such a large area of paradoxical pulsation was present in only 56 % of the posterior infarctions.

Amplitude of the systolic outward movement above the baseline varied between 4–46 mm with the present equipment. This was of the same order in both anterior and posterior infarctions, in anterior infarctions the mean was 21 mm and in posterior 18 mm (Fig. 6). No significant difference in the mean height existed between the examinations made on admission and at discharge,

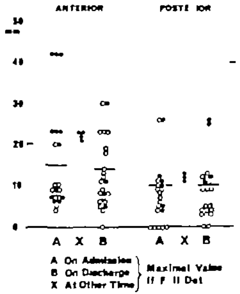


Fig. 6 Amplitudes of maximum systolic outward bulges of the KCG tracing in anterior and posterior infarctions divided into three groups according to the clinical severity The differences of the mean are not significant in total and subgroups.

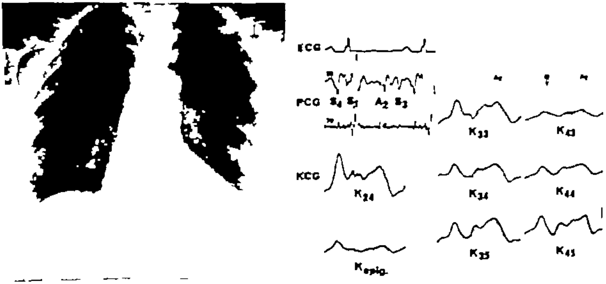


Fig. 5 Sustained paradoxical bulge present over the entire precordium in patient with an extensive anterior transmural myocardial infarction and severe left heart failure but with a normal-sized heart (330 ml/m²). Loud atrial (S_4) and ventricular (S_3) gallops are recorded in the PCG.

with only a minor tendency to lessen in the course of time. The bulge/T ratio was also similar in the anterior and posterior infarctions. Anterior infarctions in group 1 tended to cause higher bulges than the posterior infarction group 1 while groups 2 and 3 behaved quite similarly in regard to the different electrocardiographic locations of the infarction (Fig. 7).

The bulge/T ratio did not have a significant relation to acute papillary muscle dysfunction (graded 0–2) or to the occurrence of ventricular gallop on days 1 5 or 20.

The type of the maximum paradoxical pulsation was predominantly in 78% of the patients, a pansystolic bulge. The bulge reached the 2nd heart sound in 70% (35 patients) Minor abnormalities of early mid, or late systolic outward movement occurred in 22% (Table II). No patient had normal tracings throughout the serial study No clear-cut discriminating feature was found in the shape of the paradoxical movement between anterior and posterior infarctions. Three of the 4 patients with nontransmural infarctions had a minor abnormality grade of pathological movement.

Pre-ejection retraction was present in only 2 of the 50 patients. Inward retraction below the baseline occurred in 6 patients, with range of 29–80% mean 49% of the total amplitude.

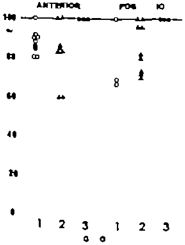


Fig. 7 The ratios of the systolic outward bulge to total amplitude of the KCG in anterior and posterior infarctions. No significant differences between the groups or between anterior and posterior infarctions were found. ○ group 1, ▲ = group 2, ● = group 3.

Table V *Sequential patterns of paradoxical movement in KCG in relation to location of infarction*

Mode of change in the pulsation	Location of infarction (no. of cases) Anterior	Posterior	Total
Present throughout			
Increased	8	9	17
Unchanged	10	4	14
Decreased	6	5	11
Present initially reverted to normal	1	2	3
Absent initially			
Developed later and persisting	0	3	3
Transient	0		2
Total	25	25	50

In the patients who died, the paradoxical motion was pansystolic in 4 and abnormal in 2 persistently in 1 a minor abnormality increased to a pansystolic bulge, and in 1 the bulge later lost its pansystolic character

Sequential changes in ventricular asynergy The serial pattern of the kinetocardiographic systolic displacement in anterior and posterior infarctions both on admission and at discharge is shown in Table V In only 5 patients, all with posterior infarction, was a normal tracing recorded on the 1st day but all these developed later a definitely pansystolic bulge (Fig. 8). Disappearance of the paradoxical pulsation was found also in only 10% (Fig. 9) Only two patients showed a transient paradoxical pulsation. In the majority of patients, 40 of the 50 (80%), the degree and pattern of the bulge showed minor variations only in the course of serial examinations. However the sequential changes were clear-cut enough to indicate the occurrence of acute myocardial damage in the two patients with complete LBBB and in 9 of the 10 with hypertension. Initial diffuse widespread bulging tended often later to localize to a smaller area as a more discrete bulge (Fig. 10).

The a wave

The maximum a/T ratio varied from 16 to 100% (mean 39%), and in 92% of cases it exceeded the normal ratio of 20% The ratios determined simultaneously were generally somewhat higher at the maximal bulge than at the apex (Fig. 11).

Table VII. *Prevalence of different grades of pulmonary venous congestion in AMI*

Day of investigation	Grade of congestion 0	1	2	3	No X-ray available
1st day no. of cases	4	.4	10	7	5
20th day no. of cases	5	24	16	3	2

The P terminal force as a measure of left atrial overloading conformed to the clinical severity of infarction, its prognosis and the radiological evidence of left heart pump failure (Fig. 12). On the first day it also paralleled the degree of the paradoxical motion, the mean P terminal force was −0 017 mmsec with normal KCG −0 024 mmsec with minor abnormalities, and −0 030 mmsec with pansystolic bulges. Further relationships of the P terminal force to other items were not analyzed in the present series, because of detailed previous studies by us (21).

Chest X-rays

Heart size The heart was enlarged in 42% of the patients during the 1st day and in 38% at discharge. Thus the frequent presence of a dynamic ventricular aneurysm, papillary muscle dysfunction or gallop sounds was an early sign of impaired left ventricular performance, occurring before cardiac enlargement in AMI. Moreover in spite of the significant aneurysmal bulging of the heart, radiological evidence of a cardiac aneurysm in the plain films was noted as bumps or protuberances of the left ventricular silhouette in 5 patients only developing acutely in 4 of the 5 The heart was enlarged in 8 of the 9 patients who died, in 3 of whom the size was normal on admission.

Pulmonary vascular congestion. The quality of even the bedside films was good, permitting assessment of the vascular abnormalities without difficulty Pulmonary vascular signs of left ventricular failure were the commonest radiological abnormalities in the serial chest radiographs taken (Table VII). Only exceptionally these were absent: totally absent in 1 of the 50 patients, absent on admission in 9% and absent at discharge in 10% of the patients with X rays available. Venous dilatation was present as the only abnormality in 50% Frank pulmonary edema occurred in 20% (Fig. 3)

The initial films generally revealed a more severe degree of left heart failure than the later ones. In 12 patients the degree of congestion was of a lower grade in the films obtained at discharge. The score remained unchanged in 13 patients, although in most of them it showed a tendency to decrease Deterioration occurred in 16 patients. Initial pulmonary edema disappeared in 5 of 7 patients. Four patients with a normal chest radiograph on admission later developed pulmonary vascular congestion, and even then of grade 1 only All signs of pulmonary venous hypertension disappeared in 4 patients. The pulmonary vascular congestion was significantly worse in the dying patients, mean score 2.56 ± 0.88 than in survivors, mean 1.51 ± 0.68 ($p < 0.01$)

Seven of the 9 patients who died displayed frank pulmonary edema in the serial chest X-ray films, persisting in the majority from admission or showing delayed development. The degree of pulmonary vascular congestion showed a statistically significant correlation to the clinical severity of AMI, the mean scores of pulmonary congestion in clinical groups 1 2 and 3 being 1.27 ± 0.70 (S.D.), 1.70 ± 0.63 and $...5 \pm 0.96$, respectively ($p < 0.01$ by F test)

Correlation to pulmonary rales All the 10 patients with radiological pulmonary edema had widespread pulmonary crepitant moist rales Dilatation of the pulmonary veins only (grade 1 congestion) was seen in both films in 23 patients, in 7 of whom basal crepitations were not noted in clinical auscultation.

DISCUSSION

In the present study of 50 patients with AMI at least some clinical signs of impaired performance of the heart as a pump were observed in all the patients. Only four patients (8 %) had a nontransmural infarction. This unusually low prevalence of nontransmural infarctions is due to the fact that mild or suspect cases of AMI were more likely to enter the usual hospital wards than the intensive care unit. In the present study the prevalence of various signs of left ventricular dysfunction were as follows: atrial gallop 100%

(audible 82%), a high wave in KCG 92% ventricular gallop 90% (audible 80%), mitral regurgitant murmur due to papillary muscle dysfunction 58% paradoxical systolic pulsation in KCG 100% (palpable 86%), pulmonary crepitant rales 70% and in chest X-rays pulmonary vascular congestion 98% frank pulmonary edema 70% and enlarged heart 4 % These prevalence values exceed those generally reported in AMI

Pathophysiological mechanisms related to ventricular dysfunction in acute ischemia and infarction

Among the pathophysiological factors that are involved in the deterioration of left ventricular function in AMI the following obviously are of the greatest importance.

1) *Ischemia of the myocardial cell.* In an isolated myocardial fiber ischemia retards the rate of tension development, maximum velocity and extent of shortening, and force of contraction (61), and the entire force-velocity curve is shifted downward and to the left. This is mediated by the prompt metabolic and structural effects of ischemia (2).

2) *Regional asynergy of the left ventricular pump unit* A normal left ventricle is structurally capable of high-pressure work, with the strong circular muscle layers contracting in an orderly sequence, both in a geometrical and temporal sense (23 44). If a myocardial region is made ischemic, the ability for muscular contraction of this region is always lost within a few seconds (17 57 59). Consequently when the intact regions of the myocardium contract normally the ischemic or necrosed area remains noncontractile, is stretched, and bulges paradoxically outwards in systole. The net contractile power and velocity generated by the entire left ventricle remain subnormal and the pump function will become depressed. Notably the hemodynamic effects of ventricular asynergy in the geometric and sequential sense, account much more for the decline of efficiency of the ejection work of the heart than the mere reduction of the contractil myocardial mass (15 18 23 28, 31 62). The validity of this concept has been testified to by marked immediate hemodynamic improvement after acute infarctectomy (22, 32).

3) *P pillary-muscle disorder* Rarely rupture of an infarcted papillary muscle occurs in AMI and then usually rapidly terminates fatally due to sudden excessive volume overload to which the infarcted left ventricle cannot adapt itself. Acute ischemic dysfunction of the mitral valve complex, without papillary muscle disruption, also imposes a varying amount of regurgitant volume load upon the already compromised left ventricle. This acute mitral regurgitation has been observed in over half of the patients with AMI (19), and experimentally even more frequently (1 17).

Infarction of the papillary muscle itself with loss of its contractile power is probably the most important factor in the interference of the delicate valve function, in association with dyssynergic movement of the supporting ventricular wall, and sometimes with acute cardiac dilatation (6, 19 20, 38, 42, 54) The size of the infarct bears little on the papillary muscle dysfunction, but rather the location of the infarct in relation to the papillary muscles (19). Owing to the distribution of the coronary arteries, the posterior papillary muscle more frequently becomes ischemic or necrosed. Further in addition to the local infarct involving the nearby papillary muscle, massive general subendocardial ischemia may not infrequently arise in the failing ventricle with a low coronary perfusion pressure (43 48). Papillary muscles are thus easily rendered ischemic, or prior dysfunction is enhanced. The spectrum of hemodynamics in acute papillary muscle dysfunction varies from insignificant to devastating regurgitation (6 19 29). The clinical outcome is also importantly governed by the function of the acutely damaged ventricular myocardium (19 20).

Hemodynamic consequences of left ventricular dysfunction in AMI

Animal experiments and, recently left ventricular catheterizations in patients with AMI have almost always shown pressure-flow alterations as a sign of impaired left ventricular performance even before overt signs of heart failure appear (1 13 14 16, 17 2., 29 46). The left ventricular end-diastolic pressure is markedly elevated in AMI owing to power failure. The Frank-Starling function curve is depressed and flattened. in spite of an elevated filling pressure the stroke volume, stroke work, and ejection fraction are all varyingly reduced, in shock down to 5–40% of normal. Characteristics of myocardial performance such as dp/dt, mean systolic ejection rate, aortic flow ac

celeration and peak velocity are likewise depressed (45) Reduced diastolic compliance of the left ventricle may be an additional factor in elevating the end-diastolic pressure, though data on the behavior of compliance in AMI are inconsistent (13 27).

The left atrial pressure is further elevated in AMI by the varying volume flow of acute mitral incompetence due to papillary muscle dysfunction into the small and nondistensible left atrium. The v peaks may rise sometimes up to 60 mmHg, enough to induce pulmonary edema (19 43).

The quantitative effects of the extent of muscle necrosis and ischemia, asynergic contraction, amount of mitral regurgitation, and also the state of the uninvolved portion of the myocardium vary individually and, accordingly the degree of impairment of left ventricular function shows wide individual variation. A spectrum exists from latent dysfunction, recognized only by loading tests (33), to severe dysfunction resulting in pulmonary edema and irreversible shock. Several vicious circles may emerge in AMI from the simultaneous occurrence of ventricular failure and dilatation, low coronary driving pressure, and functional mitral incompetence. The net result on the pump function will be determined by complex interactions of these various consequences of myocardial ischemia and necrosis.

Signs of left ventricular dysfunction in AMI

The importance of clinical and radiological bedside examinations seems to have remained less appreciated than the direct laboratory approaches in the assessment of the hemodynamic stage of left ventricular pump failure in a patient with AMI.

Atrial gallop and increased a *wave in KCG* Atrial gallop is almost invariably audible in AMI if the observation is made carefully in the left lateral position with light pressure of the stethoscope bell. It may be even easier to feel than to hear and, in analogy with this finding, the height of the a wave and the a T ratio in AMI, with only a few exceptions, are pathologically increased in KCG and often in the apex cadiogram (37).

The mechanism of pathological atrial sound and enhanced a wave in AMI is probably related both to 1) increased filling pressure of heart failure (40 55) and to 2) reduced compliance of the acutely diseased left ventricle (40 53), as well as to 3) abnormally vigorous contraction of the healthy left atrium in response to its distention by elevated left ventricular end-diastolic pressure (3) and papillary muscle dysfunction, and to 4) enhanced sympathetic drive (64).

The occurrence of atrial gallop without exception in AMI, even when uncomplicated, directly denotes very early an acute ischemic dysfunction of the left ventricle. Atrial gallop is probably the first, and rather persistent, physical evidence of ventricular dysfunction but is not useful for a quantitation of ventricular failure or for prognostic evaluations (60). Generally only the loudness of the atrial gallop showed some sequential relation to the clinical course. On the other hand, absence of atrial gallop is a strong argument against a diagnosis of ischemic chest pain, as shown also in the study of Hill et al. (24). Similarly to atrial gallop, the pathological a/T ratio on KCG was not definitely related to the severity but rather to the presence or absence of AMI, although its persistence has been suggested to imply a grave prognosis in chronic coronary heart disease (47). However the a/T ratio is greatly influenced by the pathological systolic movement in KCG so that perhaps absolute excursions of the a wave might be more informative. Electrocardiographic acute left atrial overload is of greater quantitative predictive value, and is also significantly related to ventricular failure and mitral incompetence (21).

Ventricular gallop Our finding that left ventricular gallop is audible in 90% of AMI is supported by the report of Hill et al. (24) who found it in 65%. It seems that only loud gallops have been included in earlier studies on AMI (36), or many of the commonly faint gallops have been overlooked. The mechanism of ventricular gallop is not yet exactly known, but at least it is related both to an increased ventricular inflow (39) and to a diseased myocardium (53). In AMI both myocardial damage and failure and, frequently increased inflow due to papillary muscle dysfunction are present. Probably the ventricular gallop in AMI indicates a higher filling pressure than the presence of atrial gallop (40).

With regard to the acute phase of infarction, the common association of ventricular gallop with a poor prognosis in cardiac disease is not valid in AMI, as demonstrated in the present study Ventricular gallop probably has prognostic value in infarction only if it is loud and persistently pres-

ent, with other signs of left heart failure over months following the infarction (38).

Mitral regurgitant murmur of papillary-muscle dysfunction. The clinical occurrence of acute mitral incompetence in AMI is common, being present in about half of the patients. Characteristically it has highly variable clinical features and different hemodynamic grades. These features and the consequences of acute ischemic papillary muscle dysfunction have been the subject of earlier studies by us (19 20 21). For recognition of the mostly faint pansystolic or ejection-type mitral systolic murmur careful selective auscultation is needed.

Paradoxical cardiac pulsation. The fundamental importance of ventricular asynergy in compromising left ventricular function in AMI was discussed above. The transmittance of this alteration to the precordial chest wall affords a possibility to sensitively recognize left ventricular dysfunction in ischemic heart disease by precordial motion recordings and by cardiac palpation.

Pulsations of the chest wall generated by cardiac contraction are the result of complicated changes 1) in the shape and consistency of the heart and its rotation and recoil movements, all these tending to produce a propulsion of the anterior thoracic wall during the initial part of systole, and 2) in the systolic decrease of ventricular volume during ejection, with inward retraction of the chest wall (7 10, 18). The KCG tracings are normally rather similar in the different regions of the precordium, and sustained outward motion does not occur (12, 18, 25 52). Pathological alteration in the ejection phase, contrary to that in the isovolumetric contraction phase, is readily recognized by the replacement of the normal systolic retraction by a pathological outward bulge.

The mechanism of paradoxical pulsation in AMI is obviously ventricular asynergy as pathological pulsation frequently occurs in an abnormal location, other than that in left ventricular hypertrophy or hemodynamic volume or pressure overloading, which also produce paradoxical movement (5 11 18). Moreover direct experimental observations on regional contraction disturbances in ischemia have invariably shown asynergy and ventriculographic findings have provided evidence that systolic abnormality of the precordial cardiac impulses is related to the disturbed motion of the left ventricular wall (7 15 30 54)

Our finding of a grossly pathological contraction pattern of the heart in serial KCG in all patients with AMI is in conformity with the invariable occurrence of regional ventricular bulging in experimental coronary artery occlusion, occurring in humans even in transient induced myocardial ischemia. This prevalence of 100% was noted also by Suh and Eddleman (56) in the only previous serial KCG study in acute phases of infarction in 42 patients. The principal pathological changes were: exaggerated *a* waves, diminished retraction during ejection, constantly present pathological outward motion during ejection, and often a poorly inscribed early diastolic phase of passive filling. In old myocardial infarctions abnormalities have been reported in up to 60–80% in precordial motion studies (5 47 52). Decrease or reversal of left ventricular border movement has also been frequently noted in infarctions by radiological technics of roentgenkymography and electrokymography (35 49) The frequent occurrence of acute mitral incompetence did not significantly alter the basic paradoxical motion pattern in this series (5).

On the other hand, the report of McGinn et al. (37) that paradoxical apical pulsation was observed on palpation or in apex cardiography in only 1 of 60 patients with old myocardial infarction, with or without aneurysm, seems to be a rather solitary contradictory opinion. Such findings are obviously explained by the unreliability of apex cardiography in recording absolute systolic movements. The KCG or similar method is thus mandatory for correct analysis of the systolic precordial motions (11 18, 38). The observed, practically similar, high sensitivity of KCG in recording paradoxical movement in anterior and posterior infarctions confirms the finding of Suh and Eddleman (56) and is contrary to several earlier expressed opinions (5 8, 15). It may be due to the frequent extension of infero-posterior infarctions to septal and apical areas (56), and to the posterior paradoxical pulsation pushing the whole heart against the anterior thoracic wall, as have been noted in cineradiological studies in our laboratory Otherwise the qualitative characteristics of the bulges in anterior and posterior infarctions were so similar that the KCG was unable to locate the infarction. How

ever in anterior infarctions the paradoxical pulsation occurred generally between the left parasternal area and the apex, while in posterior infarctions it was found in the apical region. This is in agreement with the study of Mourdjinis et al. (38)

The high sensitivity of KCG provides qualitative information on altered ventricular function early in AMI, even in normal-sized hearts, before the appearance of more advanced signs of left ventricular failure. This may become detectable also before definite electrocardiographic changes or serum enzyme elevations of AMI are seen. A sequentially changing pattern helps to relate the pathological findings to the acute stage of infarction even in the presence of left ventricular hypertrophy and complete LBBB (5). Maximum bulges occurring during the initial days of infarction are probably caused by large ischemic, and non-contractile (2, 41) zones surrounding the necrotic area (4), further intensified by the catecholamine surge (64). Lack of later reversion of the bulge to a normal pattern is probably due to transmural necrosis of the myocardium and persistent contraction disorder. This occurs also in an anatomical aneurysm (38)

As also noted by Suh and Eddleman, the palpatory abnormalities of ventricular contraction in AMI are correctly recognizable by careful evaluation much more commonly than was often suggested earlier (25 37 63) However it is important to realize that palpatory precordial bulges in AMI do not warrant a diagnosis of anatomical ventricular aneurysm (8) An anatomical aneurysmal sac has been noted in 3.5 to 20% of infarctions in extensive autopsy series (9 51) Dynamic disorders of ventricular contraction, recognizable only during life, are in most instances caused by infarction in a full-thickness ventricular wall and more rarely by thin-walled aneurysms (15 18, 23 41)

Radiological findings Heart volume Severe left heart failure leading to frank pulmonary edema may be present early in AMI while in this phase the heart size remains normal in the majority of the patients. The general association of enlargement of the heart with congestive heart failure cannot be applied to AMI, in which drastic cardiac failure may occur acutely without time for compensating mechanisms. In any case, a normal heart size does not rule out severe left ventricular dysfunction, as was seen in this and other studies (19 25 58).

Pulmonary vascular congestion. The dependence of progressively advancing radiological pulmonary vascular congestion on the hemodynamic severity of left heart failure has been conclusively demonstrated (19 25) The almost invariable occurrence of pulmonary venous dilatation in the present study corresponds well to the almost constant elevation of left ventricular end-diastolic pressure in AMI, pressures above 10 mmHg leading to venous congestion. The serial chest X-rays for following the degree of left ventricular failure in AMI is thus a most helpful guide and of a significant prognostic value.

Clinical Implications

Left ventricular dysfunction and power failure may be regarded as an inherent consequence of transmural myocardial infarction, even in a normal-sized heart, because of loss of contractile elements and development of asynergy Therefore, the very high prevalence of various signs of left ventricular dysfunction observed in the present series is not unexpected. Further studies are needed to evaluate the occurrence of these signs in nontransmural infarctions.

Both the diastolic filling period and the systolic contraction phase of the mechanical cardiac cycle are pathologically altered in AMI. These alterations are associated with several clinical and radiological signs, such as atrial gallop sounds, ventricular gallop, paradoxical pulsation, murmur of mitral incompetence, electrocardiographic left atrial overloading, and pulmonary vascular congestion. Even hemodynamically compensated left ventricular dysfunction occurring in AMI is recognizable by these signs (25 26, 34). On the other hand, the presence of these signs of left ventricular dysfunction as such in cases of AMI does not necessarily imply the presence of overt cardiac failure or a poor prognosis. Only electrocardiographic left atrial overloading and radiological pulmonary vascular congestion possess quantitative value in estimation of the degree of heart failure and of prognosis. In large series of AMI left ventricular dysfunction has been found to be clinically compensated in a third of the patients and shows more clear-cut clinical pump failure in two-thirds of them (6, 34 50) The pattern and grade of ventricular dysfunction must be

evaluated individually in order to select the appropriate medical, and sometimes emergency surgical, method of treatment.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

I. Clinical Symptoms and Signs

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Abstract. A material consisting of 109 patients with chronic renal failure has been examined for the presence of clinical symptoms and signs indicating peripheral neuropathy. Symptoms were reported by 84 patients (77%). Cramps and the restless legs syndrome were reported most frequently. However, several clinical observations raised doubt whether these symptoms do at all reflect peripheral neuropathy. Paresthesia (31%) and dysesthesia (18%) were the most common sensory symptoms, whereas actual pain and the burning feet syndrome were rare complaints. Clinical signs of neuropathy were recorded in 56 patients (51%). This came close to the over-all incidence in previously reported materials. Impaired vibration sense and reflex disturbances were the earliest and the most frequent findings. As a rule other signs occurred late in the course of chronic renal failure, and they were late manifestations of neuropathy as well.

During the past decade peripheral neuropathy has become generally acknowledged as an integral part of the uremic syndrome. A detailed description of the clinical picture in severe cases appeared in the early casuistic reports (1, 2, 17). Later communications have dealt with the clinical (Table III), neurophysiological (4, 6, 7, 8, 13, 14, 15, 18, 22, 25, 30), pathological (2, 10, 12, 15, 17) aspects of the neuropathy.

However, only few studies based on sufficiently large and unselected series have been devoted to a detailed analysis of the clinical features of the neuropathy, the intercorrelation of symptoms and signs, as regards the early diagnosis and the clinical severity of the neuropathy, and the relationship to other clinical variables (15, 23). Such studies may supplement the nosographic description of the clinical picture, establish the frequency and clinical significance of the neuropathy and possibly extend our understanding of which biological and pathophysiological factors might determine the clinical breakthrough and course of symptoms and signs.

The present investigation describes the frequency and character of clinical symptoms and signs indicating a dysfunction of the peripheral neuro-muscular apparatus in 109 unselected patients with varying degrees of chronic renal failure.

MATERIAL

The 109 patients were examined during the period Jan. 1966 – Aug. 1968. The material was collected among patients from the Out-patient Clinic and the Division of Nephrology, Medical Department P, University Hospital, Copenhagen. Four patients were examined during hospitalization in other medical departments of the same hospital, and 13 during hospitalization in the Medical Department C, Municipal Hospital, Aarhus. Rural and urban populations were equally represented.

Criteria of selection. The material has been collected entirely at random and as far as possible consecutively. *The following groups of patients were excluded from the material*, either in advance or when supplementary information was obtained. 1) Patients with other medical diseases, which might possibly affect the peripheral nerve system (e.g. diabetes, collagen diseases, cancer, severe arteriosclerosis). 2) Patients with pre-existing neurological disorders (syphilis, disc herniation, etc.). 3) Patients who had experienced previous neurotoxic action of drugs (e.g. furantoins and colistin). 4) Chronic alcoholics. 5) Patients who had been gastrectomized or suffered from gastro-intestinal diseases with suspected malabsorption, in particular of vitamin B_{12}. 6) Patients who had already been placed on regular hemodialysis programme. Isolated dialyses in 15 patients due to acute exacerbations of terminal uremia were, however, not considered sufficient reason for exclusion.

Age and sex. The material included 49 women and 60 men. The age ranged from 13 to 73 years, the 10-year age groups between 20 and 60 being approximately of

Table I *Classification of the primary renal disease in 109 patients with chronic renal failure*

The figures in parentheses refer to the number of histologically verified diagnoses

Chronic interstitial nephropathy ("chronic pyelonephritis")	40 (18)	
Chronic glomerulonephritis	38 (36)	
Other chronic renal diseases	31 (14)	
Polycystic kidneys		6 (3)
Papillitis necroticans		6 (1)
Nephrosclerosis		7 (3)
Hydronephrosis		2 (0)
Medullary sponge kidney		3 (3)
Alport syndrome		1 (1)
Nephrocalcinosis		1 (1)
Unclassified chronic nephropathy		5 (2)
	109 (68)	

equal size The average age for the whole material was 40.1 years, and was almost identical for women and men (40.0 and 40.4 years respectively).

The primary renal disease The nature of the primary renal disease was established according to usual clinical criteria. The clinical examination was supplemented by histological examination of the kidney tissue in 68 patients (obtained by renal biopsy in 32, by bilateral nephrectomy in 24, and at autopsy in 12). The diagnoses appear in Table I. "Unclassified chronic nephropathy" comprises patients in whom histological diagnosis of the primary renal disease was impossible due to maximally contracted kidneys. The two main groups, chronic interstitial nephropathy (chronic pyelonephritis) and chronic glomerulonephritis, are almost equally represented and comprise 72 of the material. The sex ratio, females males, in chronic interstitial nephropathy is 26/14 and in glomerulonephritis 12 26 ($\chi^2 = 3.70$, $p > 0.05$). The average age of patients in the two groups is 45.0 years against 33.2 years ($p < 0.001$). The mean duration of recognized azotemia at the time of the investigation is about one year longer in patients with interstitial nephropathy (4 months) than in patients with glomerulonephritis (29 months).

Renal function and degree of azotemia. The renal function as evaluated by measurement of the endogenous creatinine clearance (C_{cr}), which was determined as the mean value of at least three consecutive 24-hour periods and expressed in ml min. C_{cr} ranged from 80 to 0 ml min, the median value being 8 ml min. The degree of azotemia was expressed by the serum levels of urea (range 0.43–5.00 g l, median value 1.50 g l) and creatinine (range 1 345 mg l, median value 86 mg l) recorded on the day of examination.

METHODS

The present study was restricted to information obtained from neurological bedside examination. A number of patients were examined at various stages of progressive renal failure, but for the analysis of the frequency of neurological findings each patient was counted only once. In patients with severe renal failure this would as a rule represent the last examination before the institution of regular dialysis, renal transplantation, or death.

The history comprised thorough inquiry into all symptoms related to the peripheral neuro-muscular system. Cramps were distinguished from the restless legs syndrome. An attempt was made to get precise picture of the extent of muscular weakness, but this was often unsuccessful, due to the general and often profound physical tiredness, so prevalent in these patients. The patients were requested to give detailed description of the character and localization of sensory symptoms, the time of onset, changes in intensity as well as their significance for their normal activity

Objective findings. The deep tendon reflexes (biceps, triceps, patellar and ankle) were considered abnormal only when definitely weak or absent under good relaxation and reinforcement. The vibratory perception threshold (VPT) was determined with an electromagnetically driven vibrator (Biothesiometer). The vibration sense was "impaired" when the VPT exceeded twice the standard deviation of the mean value for the age of the patient. The reproducibility and the range of normal variation has been reported in a separate study (19). The cutaneous sensibility to light touch and pinprick, as well as the functional state of the muscles, were tested by conventional technique.

An endeavour was made to render the clinical evaluation of the peripheral neurological state in the patients entirely unprejudiced by the severity of the renal failure and by other clinical variables. Symptoms and signs of autonomic neuropathy (11), or of cerebral disorders, have not been recorded systematically and are therefore disregarded in this study In about 70% of the material the clinical examination was followed by determination of the sensory and motor nerve conduction velocity. Information thus obtained has not been considered in the present context. An analysis of the results will appear in a subsequent publication.

RESULTS

Table II summarizes the frequency of single symptoms and signs among the 109 patients.

Relevant descriptions of motor and sensory symptoms were given by 84 patients (77%). Irritative muscular symptoms (cramps, restless legs) and paresthesia were the dominant complaints.

Objective neurological findings were present in 56 patients (51%). Impaired vibration sense and abnormal reflexes were by far the most frequent signs of peripheral nerve dysfunction, while other objective criteria (pareses, muscular atrophies, cutaneous hyposensibility) occurred in only 15% of the material.

Subjective Complaints (Symptoms)

Cramps and restless legs (73 patients, 67 %) were described in almost unanimous terms by all patients, and the two symptoms were as a rule easily distinguished. Thus 18 patients with the restless legs syndrome had never experienced cramps, whilst the opposite was true in 30 patients. Twenty five patients described both symptoms. The restless legs in these cases tended to appear at a later stage in chronic renal failure, while cramps had been present, usually with symptom-free periods varying in length from months to years. The two symptoms differed clinically in several respects.

Cramp were often painful and developed mostly during the night. They tended to be localized to single muscles or muscle groups, usually in the lower extremity while the upper extremity was affected more rarely although this was the *only* localization in a few patients. The extremity became locked in an awkward position, but cramps often loosened when the extremity was redressed. Many patients knew certain tricks to get rid of the cramps.

Patient 77

A 59-year-old man with chronic interstitial nephropathy developed intermittent nocturnal cramps in the calves and toes tw years before his death. During the last year he became gradually more troubled by restless legs in the evenings. In the same period, but more rarely he experienced sudden flexion cramps in the ulnar fingers in both hands. The fingers had to be redressed by force before the cramps subsided. Having experienced the latter couple of times hile driving his car, he gave up driving. Apart from elevated vibratory perception threshold on toes and malleoli, he did not develop other clinical evidence of neuropathy

Restless legs. Many patients had to restrict their social activities because of an irresistible restlessness in the calves. This often developed in the afternoon or in the evening, whenever they relaxed in a chair One patient could no longer take part in meetings, and several preferred to refuse invitations. A 28-year-old femal (patient 48) had to give up her job as naval radio operator Insomnia, often for several hours, was a common complaint, and the patients knew of no other remedy than to walk around. Although rarely painful, restless legs thus caused a considerably greater strain on the patients than did the short-lasting attacks of cramps. The upper

Table II *Clinical neurological findings in 109 patients with chronic renal failure*

A. *Symptoms*	84 (77)	
I. Motor	76 (70 %)	
Restless legs		43
Cramps		55
Muscular weakness		10
II. Sensory	44 (40)	
Paresthesia		34
Dysesthesia		20
Pain		6
Burning feet		7
B. *Signs*	56 (51 %)	
Pareses	15 (14)	
Muscular atrophies	13 (13 %)	
Abnormal reflexes	31 (28 %)	
Achilles		30
Patellar		23
Biceps or Triceps		7
Impaired vibratory perception	42 (38)	
Pulp, big toe		37
Medial malleolus		34
Pulp, thumb		10
Hypesthesia or hypalgesia	17 (16)	

extremity was never affected in these patients, the localization was usually bilateral in the single attacks, and proximal muscle groups were involved more frequently than in cramps. In some patients the restless legs syndrome became the most distressing part of the uremic syndrome, as exemplified by the following case history

Patient 92

A 35-year-old lorry driver ith chronic interstitial nephropathy became increasingly troubled by restlessness in the legs during the last tw years before his death. He had to give up his job partly because of severe and chronic tiredness due to severe insomnia, as he rarely slept for more than one hour at time, sometimes only tw to three hours per night. His wife preferred to sleep in separate room, due to his endless moving or alking around. During the last six months before his death, he developed severe and rapidly progressive polyneuropathy

Muscular weakness. Due to the sometimes excessive tiredness, which is part of the terminal uremic syndrome, nearly all patients experienced a diffuse and progressive physical weakness, which made them incapable of performing normal work of even moderate degree. Many patients complained of difficulty in walking, and especially in negotiating stairs. Weakness in isolated muscle groups, on the other hand, was a rare complaint, which was recorded in only 10 of the 109 pa-

tients. It often started with weakening in the toes, and nine of the patients described unsteady gait, especially on uneven ground, with reduced control of one or both feet. One patient (patient 90) had to walk backwards over doorsteps, another had difficulties in stretching his knees even when climbing shallow stairs. Both presented objective evidence of quadriceps paresis. Four patients reported that things dropped out of their hands. Two of them, both with severe neuropathy could no longer eat unaided. As a rule muscular weakness was preceded by other symptoms or signs. Patient 47 presents an exception.

Patient 47

A few weeks after the institution of regular dialysis a 27-year-old female with chronic glomerulonephritis became alarmed by the fact that she could no longer move her toes. Only 2 months earlier a clinical examination had shown completely normal findings, and she had denied every symptom (this is the examination referred to in the present study). At that time, however the motor conduction velocity in her left peroneal nerve was clearly reduced (31 m/sec). Within a few months following this initial symptom she developed the severest and most progressive neuropathy seen in the present material.

Paresthesia (34 patients, 31 %) Paresthesia was characterized in various ways as a pricking, tingling, effervescent ("like fizzy drinks") sensation, sometimes painful especially when located to the soles sometimes causing a warm sensation in the skin (which was not considered to be equivalent to the burning feet syndrome). Except in terminal uremia, but even then, paresthesia usually caused little trouble and was rarely reported spontaneously Paradoxically in a few patients the presence of the symptom in the uremic state first became clearly recognized when it disappeared after a renal transplantation. The localization was predominantly distal, fingers and toes being affected with almost equal frequency (20 and 26 patients respectively) With few exceptions the cutaneous areas involved were diffusely outlined. Paresthesia was definitely an early manifestation of a developing peripheral neuropathy but the time of onset in the course of renal failure varied considerably The onset was often well defined and as a rule related to a sudden deterioration in the clinical state of the patient, accompanied by other uremic manifestations. The paresthesia might be intermittent and often temporarily disappeared as a result of intensified treatment (dietary restriction, correction of electrolyte disturbances). This was particularly striking in some patients after one or two dialyses. Persistent paresthesia despite treatment tended to indicate a progressive course of the neuropathy

Patient 7

A 41-year-old carpenter with polycystic kidneys observed intermittent paresthesia, localized to the fourth and fifth fingers, about four years prior to renal transplantation, i.e. when the renal failure was still moderate (C_{cr} about 20 ml/min). The paresthesia was initially unilateral and was brought on by pressure (using a screwdriver) but it soon became bilateral and spontaneous, projecting towards the elbow along the course of the ulnar nerve. One year later he complained of paresthesia in the other fingers as well, and in both legs ("as if fizz was being injected into my veins"). Later dysesthesia in the feet supervened, accompanied by lancinating pain and nocturnal burning feet. In the terminal stage he reported unsteady gait, but he still remained free of objective clinical findings until a few weeks after the institution of regular dialysis, when he developed severe sensory-motor polyneuropathy

Dysesthesia (20 patients, 18 %). Dysesthetic sensations were described in the fingertips (6) and feet (16) This was the only symptom in four patients, otherwise preceded by paresthesia. Most patients became gradually accustomed to the strange feeling of numbness" rubber plates or cotton-wool" beneath the soles, of walking in water" or on cushions or of felt between the toes or fingers, but the symptom rarely disappeared entirely as a result of intensified treatment, including dialysis. Dysesthesia occurred relatively late in the course of renal failure as well as in the development of peripheral neuropathy

Pain (6 patients, 5.5 %). With one exception (patient 7), pain always afflicted patients with a well established polyneuropathy as a late symptom. Darting pain along the peroneal nerve from foot to knee was described by four patients. One had irradiating pain in the thigh, which was not referable to disc herniation. Another complained of pain along the course of the ulnar nerve Diffuse pain in the feet brought on by pressure was reported by only two patients. Thus in this material pain was a rare symptom, usually not very pronounced, and as a rule intermittent

The burning feet syndrome (7 patients, 6 %) The common complaint was severe insomnia, due to the burning sensation in the feet. Two of the

patients were middle-aged women (53 and 55 years), who had a mild nephropathy (C_{Cr} about 30 ml/min). Only four patients presented clinical signs of neuropathy

Objective Findings (Signs)

Abnormal deep tendon reflexes (31 patients, 28%). In nearly all patients reflex disturbances were bilateral, and with one exception the Achilles reflex was always involved.

Patient 90

At the first examination this patient, 42-year-old man, had very weak patellar reflexes and moderate pareses of the quadriceps muscles. The latter became clinically prominent during the subsequent dialysis period, but then as part of polyneuropathy including absent Achilles reflexes as well.

The patellar reflexes were affected in 23 patients, while the triceps and biceps reflexes were only missing in seven patients, all of whom had terminal renal failure In six of the patients this was part of a moderate to severe neuropathy while in the seventh (patient 97 female, 24 years) deep areflexia was the only neurological finding. After a renal transplantation all her reflexes were gradually reestablished.

Impaired vibration sense (4_ patients, 38%) was the most frequent single finding. The pulp of the big toe and the medial malleolus were the earliest and most severely affected test spots, whereas the vibratory perception was rarely impaired on the upper extremity notwithstanding the presence of other clinical signs of neuropathy A statistical analysis of the VPT will appear in separate paper (20)

Pareses atrophies (15 patients, 14%). The earliest clinical indication of muscular dysfunction was paresis of the dorsiflexion of the big toe, and atrophy of the ext. dig. brevis muscle (13 patients), indicating an affection of the peroneal nerve. Retained dorsiflexion of the lateral toes served as a valuable control. Three patients presented symptoms and signs of a bilateral quadriceps paresis, but the proximal muscle groups were otherwise rarely involved, and always as part of a clinically pronounced polyneuropathy Pareses and atrophies of the small hand muscles, especially of the hypothenar group, was recorded in five patients. This was an isolated finding in a 13-year-old female (patient 10) with terminal uremia, but otherwise part of a pronounced poly neuropathy

Hypesthesia and/or hypalgesia (17 patients, 16%). They never exceeded the glove and stocking area, but were often confined to only one or more fingers or toes. Severe sensory loss was observed in only three patients, two of whom presented the severest polyneuropathy seen in this material Rather than *hypo*sensibility several patients showed *hyper*sensibility to touch and pinprick. This was, however difficult to distinguish from the normal, but was prominent in at least two patients with peroneal palsy

With few exceptions motor and sensory dysfunctions were late manifestations in the course of chronic renal failure.

DISCUSSION

In general the symptoms and signs did not differ in character from those in other neuropathies. There was a remarkable tendency among the uremic patients to neglect relevant symptoms, either because they had gradually become accustomed to them, or more often because neurological complaints had become overshadowed by other uremic symptoms. Close contact with the patients during their hospitalization proved valuable, since it often happened that patients turned up by themselves after the first examination to supplement their own descriptions. Hence the true incidence of neurological symptoms in uremia is probably more apt to be underestimated than in other types of neuropathies.

The early subjective complaints of neuropathy in uremic patients are commonly characterized as "burning sensations in the feet" and "painful, tender soles symptoms which are well known in, for instance, the diabetic neuropathy Although certainly part of the clinical picture of well pronounced neuropathy they were extremely rare as early complaints in the present series, although specifically asked about in each patient. This is in keeping with the detailed descriptions in the early casuistic reports (2, 17) and Asbury et al. (2) directly comment that symptoms of neuropathy were far less painful in uremic patients than in diabetic patients. Jennekens (15) recorded the burning feet syndrome in only five of his 82 patients, and all five had severe neuropathy A simi-

Table III *Reported frequencies of neurological findings in unselect series of patients with chronic renal failure*

Authors	Year	No. of observations	Signs of neuropathy	Sensory				Motor	
				Paresthesia	Dysesthesia	Vibratory percept.	Hypesthesia Hypalgesia	Reflex impairment	Pareses Atrophy
Versaci et al.	1964	24	14 (58%)	(11)[c]				(10)	
Tenckhoff et al.	1965	25	6 (24%)	—[d]	—	6	4	0	0
Konotey-Ahulu et al.	1965	20[a]	15 (75%)	(15)		8	—	15	2
Coomes et al.	1965	32	4 (13%)	2	—	—	2	—	1
Tyler & Gottlieb	1965	(58)[b]	24 (41%)	—	—	—	—	—	—
Callaghan	1966	25	12 (48%)	(5)	—	9	6	11	8
Dobbelstein et al.	1968	40	21 (52%)	6	—	16	2	13	2
Robson et al.	1968	30	5 (17%)	—	—	—	—	—	—
P kkenberg et al.	1968	28	3 (11%)	—	—	—	—	1	—
Jennekens	1969	82	35 (43%)	16	12	27	—	31	—
Prill	1969	133	— ?	—	—	—	—	—	—
Present material	1970	109	56 (51%)	34	20	41	17	31	15

[a] All terminal uremia. [b] Ref. Robson et al. [c] Not specified. [d] Not indicated.

lar observation was made by Prill (23) in 133 patients.

Cramps and restless legs were by far the most frequent of all complaints. Also they were the earliest symptoms from the peripheral neuromuscular system during the course of progressive renal failure. It still remains unsettled, however how these symptoms are related, clinically as well as pathophysiologically to peripheral y Some authors consider them to be important and early indications of a neuropathy (3 5 28) As a result of the demonstration of a decreased motor conduction velocity in four patients with restless legs without other clinical evidence of neuropathy Callaghan (5) suggested that restless legs might be an early sign of peripheral nerve damage. His view was shared by Tyler (28) Jennekens (15) pointed to the gamma-motor neuron system as the site of affection.

In several respects these symptoms do in fact differ from other clinical manifestations of neuropathy in progressive renal failure. They are frequently reported by patients with mild renal failure, and they tend to be more intermittent in character than other symptoms. They may become severely pronounced in patients who do not present other evidence of neuropathy but, vice versa, clinical signs and symptoms as well as a significant reduction in motor conduction velocity are not rare findings in patients who have never experienced irritative muscular symptoms. Clinical experience suggests a close relationship between these symptoms and disturbances in the electrolyte concentrations and the acid-base status. Symptoms were often relieved when such disturbances were corrected and rarely reoccurred as long as normal conditions were maintained. During hemodialysis, muscular symptoms were often brought on by too rapid reduction of the extracellular fluid volume or when patients were dialysed against low dialysate sodium concentrations. Such attacks were usually relieved by the injection of concentrated sodium chloride into the blood line. Thus one is left with the impression that these symptoms do not necessarily constitute an integral part of the peripheral neuropathy in uremia. A correlative analysis, taking into account clinical biochemical, and neurophysiological data, is, however still lacking.

The reported frequencies of clinical signs of neuropathy differ widely (Table III). This is due mainly to variations in the composition and size of the material. The frequency in the present series of 51% (95% limits = 41–60% (9)) comes close to the over-all incidence of clinical neuropathy in most unselect series. Paresthesia is generally considered an early symptom, and reflex impairment and loss of vibratory perception are the earliest objective findings in most series. Other clinical findings tend to occur late in chronic renal failure usually in their terminal stage only Signs were often slightly pronounced and might easily escape attention unless specifically looked for The distal localization is generally approved, and

this study confirms the experience of Jennekens (15) and Prill (23) that the extensor digitorum brevis muscle (dorsiflexion of the big toe) seems to be the predilection site of early affection of the muscles.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

II. Intercorrelation of Clinical Symptoms and Signs and Clinical Grading of Neuropathy

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Abstract. Clinical symptoms and signs of peripheral nerve dysfunction in 109 patients with chronic renal failure have been intercorrelated. Paresthesia was the symptom best correlated to signs favouring neuropathy, whereas cramps and restless legs occurred equally frequently in patients with and without signs. The discriminant power of each single symptom and sign for the detection of peripheral neuropathy was analysed. The results indicate that 91% of all patients presenting neurological findings would be detected when any one of the following three criteria are present alone or in combination: paresthesia, impaired vibratory perception, and reflex disturbances. A grading of clinical findings into three groups (eight classes) is presented, according to the number of symptoms and signs, severity and extent of neurological affection.

The criteria selected for the definition of a neuropathy have constantly been a matter of dispute. The establishment of the diagnosis is essentially determined by the extent and reliability of the examinations applied. Thus the growing application of neurophysiological methods during the past 15 years has widely changed the concept of the true incidence of peripheral nerve dysfunction in several disease entities by demonstrating the presence of the so-called "subclinical neuropathy" in a considerable number of clinically intact patients.

The concept of normality has thus become more differentiated, and consequently the statement "absence of neuropathy" has become less meaningful unless it is clearly indicated at which level the examination was performed (e.g. absence of *clinical* signs or lack of *neurophysiological* evidence of neuropathy). Vice versa, the clinical term "polyneuropathy" should be reserved for the clinical syndrome, which in most instances represents the late stage of a more or less prolonged progressive nerve dysfunction.

In a previous paper (2) the number and character of clinical symptoms and signs of peripheral nerve dysfunction were described in 109 patients with chronic renal failure. No single symptom or sign appeared to be particularly indicative of the presence of a neuropathy. *A clinical neuropathy was therefore taken to be present whenever a peripheral nerve dysfunction was demonstrable by objective clinical findings (signs).*

From the clinical experience not all of the recorded subjective complaints appeared to be relevant for the presence of a neuropathy. It is therefore the first purpose of the present paper to analyse the intercorrelation of symptoms and signs.

A considerable overlapping of symptoms and signs was found. This is due, in all probability, to the fact that the development of clinical neuropathy describes a continuum of impairment of various functions. Thus in an unselected material a pattern of early and late clinical manifestations will be present. However, despite the fact that clinical symptoms and signs are non-numerical variables, the diagnostic significance of the single variable can be estimated according to the principles of a discriminant analysis. It is the second purpose of the present paper to attempt such an analysis by stepwise ranking of the discriminant power of each symptom and sign.

Finally an attempt has been made to grade the bulk of clinical information into a scale of severity of the neuropathy. This may serve as a basis for a classification of the material.

Table I *Correlation between single symptoms and signs of clinically manifest neuropathy in 109 patients with chronic renal failure*

Symptoms	No. of pat.	Signs of neuropathy (n = 56)	No signs of neuropathy (= 53)	χ^2	p
Cramps	55	31	24	0.74	N.S.
Restless legs	43	24	19	0.30	N.S
Paresthesia	34	25	9	8.45	< 0.005
Dysesthesia	20	16	4	6.23	< 0.02
Muscular weakness	10	9	1	4.98	< 0.05
Burning feet	7	4	3	(0.01	N.S.)
Pain	6	5	1	(1.42	N.S.)

valuable for comparative purposes as well as for the long term follow-up of the clinical course.

RESULTS

Intercorrelation of Symptoms and Signs

The relationship between symptoms and signs of neuropathy *appears from Table I. Cramps* and rest less legs were reported by an almost equal number of patients with and without clinical neuropathy (56 and 44% respectively) The closest correlation was found between paresthesia and positive signs of neuropathy ($\chi^2 = 8.45$ $p < 0.005$). However the clinical examination failed to demonstrate signs in nine of the 34 patients (26%), who complained of paresthesia. On the other hand, paresthesia was denied by 31 patients who presented signs. The neuropathy was mild in 20 of these 31 patients (classes III–IV see below), and moderate in 10 (classes V–VI). One patient developed a severe neuropathy within a week.

Table II. *Coincidence between signs of neuropathy in 56 of 109 patients with chronic renal failure*

A. *Single signs*	No. of pat.	Clinical signs other than A Present	Absent	
Impaired vibration sense	42	27	15	64
Reflex disturbances	31	23	8	74
Pareses and muscular atrophies	16	13	3	81
Hypesthesia and/or hypalgesia	17	17	0	100

Dysesthesia and muscular weakness were rarely reported by patients who did not present signs, and these symptoms were definitely late manifestations of neuropathy The lack of correlation between pain and burning feet and signs is inconclusive, due to the low number of observations.

The coincidence between signs of peripheral neuropathy appears from Table II. Pareses and muscular atrophies and impaired cutaneous sensibility were rarely observed as isolated findings. A significantly elevated vibratory perception threshold (VPT) was an isolated sign in 15 patients, six of whom reported other sensory symptoms. The VPT was within normal range in 1 patients despite other signs of neuropathy None of these patients had other sensory defects, but four complained of paresthesia. In eight younger patients (17–39 years) with severe uremia, reflex *disturbances were the only signs. Three of them* had sensory symptoms. In contrast, _5 patients had normal reflexes despite the presence of other signs.

The discriminatory power of single symptoms and signs

The validity of single symptoms and signs to discriminate patients with peripheral nerve dysfunc tions is graphically demonstrated in Fig. 1 When the diagnosis is based on the presence of signs only (Fig. 1 *A* 56 patients) 75% of the patients would be detected, using the VPT alone When *reflexes were tested in addition, the discriminatory* power was raised to 93% Extension of the ex amination to cover cutaneous sensibility and the functional state of peripheral muscles only resulted in the discovery of another four patients (7%) Three of these (patient 10 female, 13 years old patient 98 female, 34 years old patient 100 male, 22 years old) had "mild" neuropathy with minor and isolated pareses. The first died a few months later the other two were transplanted shortly after the examination. The fourth patient (patient 64 male 5 years old) had a clinically moderate neuropathy with peroneal palsy His reflexes were retained, but unfortunately he was one of the two patients in whom the vibration sense was not tested.

When symptoms (other than cramps and rest less legs) were included in the diagnostic criteria,

the group was extended to comprise 68 patients (62% of the material, Fig. 1 *B*). Paresthesia alone accounted for nine of these 12 additional cases (13% of the 68 patients). The isolated occurrence of other symptoms added only three new cases (5%) in all.

Hence for the detection of subjective and objective neurological dysfunctions the simple variables, vibration sense, reflexes, and paresthesia, were discriminant for 62 of the 68 patients (91%).

Clinical Grading of the Neuropathy

The main classification was established between the 56 patients (51%) who presented signs of peripheral nerve dysfunction and those 53 who did not (49%). Ideally a more differentiated grading should consider the type and number of functional impairments, the extent of the affection (i.e. distal/proximal localization, involvement of lower/upper extremities), and the severity of

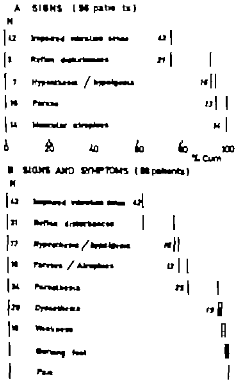


Fig. 1. The discriminative effectiveness of single signs (*A*) or signs and symptoms (*B*), ranked in order of frequency.

Table III. *Clinical grading of neuropathy*

Group	Class	Definition
A. No neuropathy	0	No symptoms
	I	*Cramps and/or restless legs only*
	II	Other symptoms than in I
B. Mild neuropathy	III	One sign only
	IV	Symptoms in II + one sign only
C. Moderate to severe neuropathy	V	Two or more signs, lower *or* upper extremities
	VI	Two or more signs, lower *and* upper extremities
	VII	*Severe symptoms and signs, lower and/or upper extremities*

neurological dysfunctions. This would, however result in a multitude of minor classes. For a realistic clinical approach the fulfilment of the following criteria was demanded.

The grading should 1) be based on the neurological records only i.e. unprejudiced by other clinical, biochemical, neurophysiological or biological (sex, age) information. 2) It should not be unreasonably differentiated in order to retain a sufficient number of patients in each class. 3) The limitation of each class should be simple and unequivocal, and as far as possible be uninfluenced

Table IV. *Distribution f symptoms and signs according to the clinical grading f neuropathy*

Group	A			B		C			Total
Class	0	I	II	III	IV	V	VI	VII	
No. of pat.	18	23	12	16	10	12	13	5	109
Per cent	16	21	11	15	9	11	12	5	100
Pain	—	—	1	—	—	1	—	4	6
Burning feet	—	—	3	—	3	—	—	1	7
Weakness	—	—	1	—	1	3	1	4	10
Dysesthesia	—	—	4	—	2	3	6	5	20
Paresthesia	—	—	9	—	6	8	7	4	34
Pareses/muscular atrophy	—	—	—	2	1	4	4	5	16
Hypesthesia/hypalgesia	—	—	—	—	—	3	9	5	17
Reflex disturbances	—	—	—	5	3	11	7	5	31
Impaired vibration sense	—	—	—	9	6	11	12[a]	4[a]	42
Cramps	—	14	9	8	6	8	6	4	55
Restless legs	—	15	4	7	3	5	6	3	43

The vibration sense was not tested in one patient.

Table V *The course in patients with monosymptomatic neuropathy*

PRF = Progressive renal failure. RDT = Regular dialysis treatment. RAT = Renal allotransplantation.

	No. of pat.	Previous normal findings	Symptoms	Course: Unknown	(Died)	Remission PRF	Remission RDT	Remission RAT	Progression PRF	Progression RDT	Progression RAT
Paresthesia without signs	9	9	3[a]	2	(2)	2	3	0	1	1	0
Impaired vibration sense only	15	—	6	8	(3)	0	1	1	2	3	0
Reflex disturbances only	8	8	3	4	(4)	0	3	1	0	0	0
Total	32	17	12	14	(9)	2	7	2	3	4	0

[a] Other than paresthesia.

by subjective judgment. 4) The grading should represent a real and continuous progression in the severity of clinical findings, which implies that the most affected patient in a lower class should be less affected than in the least affected patient in the succeeding class.

Three groups (eight classes) were defined as shown in Table III. Patients complaining only of muscle cramps or restless legs were separated from those without complaints at all. A single positive sign was considered to be more serious than neurological symptoms only although the clinical impression might sometimes suggest the opposite. Severe neuropathy confined for instance, to the lower extremities was regarded as more serious than mild or moderate signs in the lower as well as the upper extremities.

Table IV demonstrates the distribution of clinical findings in the eight classes.

The significance of monosymptomatic neuropathy

In 32 patients paresthesia without signs (9 patients) or impaired vibration sense (15 patients) and reflex disturbances (8 patients) were isolated findings (Table V). Paresthesia was a newly developed symptom in all nine patients, and brisk reflexes had been demonstrable at earlier admissions in all eight patients, whereas the vibration sense had not been tested previously in any of the 15 patients.

The prognostic significance of these findings remained unclarified in 14 patients who were seen only once. Nine of them died within a few months. In 11 patients a remission of symptoms or signs was recorded. In two the remission occurred spontaneously in nine after the institution of regular dialysis or after renal transplantation. Seven patients showed progression in the clinical neuropathy either during progressive renal failure or during regular dialysis treatment.

DISCUSSION

The lack of correlation between cramps and/or restless legs and objective findings favouring peripheral neuropathy adds further evidence to the clinical impression (2) that these symptoms are irrelevant in the present context. Hence their presence was disregarded in the clinical evaluation of the peripheral nerve function. The dysesthesia and muscular weakness, as well as objective neurological dysfunctions of the peripheral muscles and the cutaneous sensibility were rarely encountered as isolated findings. This is compatible with the clinical experience that these phenomena occurred late in the course of peripheral neuropathy (2). Hence these criteria were badly suited for the early detection of a neuropathy.

This conclusion was strongly supported by the discriminant analysis which demonstrated that less than 10 of the patients would escape detection when the incidence of clinical nerve dysfunction is screened by the use of simple and easily applicable criteria such as paresthesia, impaired reflexes, and vibration sense. Moreover from the case histories of those patients who were missed,

no serious disadvantage of this simplification was apparent.

Unfortunately this material did not provide conclusive information on the diagnostic and prognostic significance of isolated symptoms or signs. This was due to the fact that the natural course of a presumed neuropathy was either not followed (5 patients), was interrupted by the death of the patient (9 patients) or was interfered with by the institution of dialysis or by transplantation (13 patients).

It must be emphasized that the introduction of a quantitative determination of the VPT has undoubtedly made possible the detection of more cases with mild impairment of the vibration sense than would have been possible when using a tuning fork. In addition, this modification proved to be particularly valuable in following the course of the neuropathy in the single patient. It remains to be established to what extent the incidence of peripheral neuropathy is overestimated by including an elevated VPT among the diagnostic criteria. Dobbelstein et al. (1) recorded a significant reduction of the motor conduction velocity in the legs in 13 out of 16 patients with impaired vibration sense (tuning fork). In another two patients the conduction velocity was low borderline, while it was not determined in the last. In 21 patients with normal vibration sense the conduction velocity was reduced in 11 according to their criteria. The difference was not statistically significant ($\chi^2 = 3.21$ $0.10 > p > 0.05$), but this was due to the number of false negative results rather than to the number of false positive recordings of impaired vibration sense.

The clinical significance of the peripheral neuropathy in chronic renal failure can only be evaluated when the number and severity of symptoms and signs are properly graded. Hence, among the 56 patients (51 %) presenting signs of neuropathy at most 18 (16 % of the total material) attracted clinical attention due to the severity of clinical manifestations, and in only five of these patients did the neuropathy reach an invalidating degree at the time of examination. These figures compare well with those reported by Dobbelstein et al. (1). Twenty-one of their 40 patients (52 %) presented clinical signs of peripheral nerve dysfunction, yet only six patients (15 %) were stated to have "clinical neuropathy". Thus the common impression that peripheral neuropathy constitutes a considerable clinical problem hardly applies to an unselect group of uremic patients. A further reduction of the frequency of clinically significant neuropathy presumes an early detection of neurological manifestations, and any evidence of impending progression should be given a high priority in deciding on active treatment, preferably renal transplantation.

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THE PERIPHERAL NERVE FUNCTION IN CHRONIC RENAL FAILURE

III. A Multivariate Statistical Analysis of Factors Presumed to Affect the Development of Clinical Neuropathy

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Abstract. A material of 109 patients with varying degrees of chronic renal failure has been divided into two groups according to the presence (56 patients) or absence (53 patients) of clinical signs of neuropathy. A relationship between the primary renal diseases and neuropathy could not be demonstrated either in females or males. In both groups the two sexes were comparable with respect to age, renal function, and degree of azotemia. There was a significant predominance of males among patients with neuropathy. A multivariate discriminant analysis comprising age, creatinine clearance, serum creatinine and urea, and serum sodium and potassium levels, failed to demonstrate any significant difference between females with and without neuropathy. In male patients a significant difference was present. The creatinine clearance and the age were ranked as the most significant discriminating variates. The combined use of all six variates did not improve the discriminative effectiveness, but the creatinine clearance could be replaced by the serum creatinine concentration. It is concluded that the biological variates, sex and age greatly influence the breakthrough of clinical signs of neuropathy at any given degree of renal failure.

It still remains an unsettled and challenging problem that some patients with chronic renal failure contract clinical neuropathy while other patients do not, although they are apparently equally uremic. Little information is available from the literature on this topic.

It is a common clinical experience that the incidence of neuropathy increases with advancing degree of azotemia, and a significant correlation has been demonstrated between the deterioration of the motor conduction velocity and the serum creatinine level (2). A critical degree of azotemia for the appearance of clinical neuropathy has often been discussed (4, 13, 23, 28). However urea hardly exerts any neurotoxic action in clinical concentrations (18), and Giovannetti et al. (9) failed to introduce electrophysiological or histopathological signs of neuropathy by intoxicating dogs with creatinine. The same group presented experimental confirmation of an earlier observation by Mason et al. (17), that methylguanidine exerts a direct neurotoxic action. Methylguanidine, a precursor of creatinine, is a strong enzyme inhibitor which has been shown to accumulate in chronic renal failure (10), but which has not yet been systematically determined in large patient materials.

The significance of the *duration of azotemia* is difficult to assess. The deterioration of the kidney function is not a linear function of time, and many patients did not appear for medical examination before uremia had reached an advanced stage. Previous authors have not succeeded in demonstrating any relationship between the duration of azotemia and the incidence of clinical neuropathy (13, 28, 30). Clinical experience often leaves the impression that the *rate of deterioration in kidney function* is of greater significance (13). Finally in quite a number of patients the actual breakthrough of clinical manifestations often appears to be elicited by *factors which may be unrelated to renal function and the degree of azotemia*, e.g. infections, operations, or simply prolonged bed rest (28).

The concept of a progressive segmental demyelinization as the fundamental histopathological process in the development of the peripheral neuropathy directly brings into focus the pre-

Table I *Primary renal disease in 109 patients with and without peripheral neuropathy*

Clinical neuropathy	Sex	Primary renal disease: Chronic interstitial nephropathy	Chronic glomerulo-nephritis	Other renal diseases	All groups
I Present	♀	12	4	3	19 (34 % of I)
	♂	10	17	10	37 (66 % of I)
II. Absent	♀	14	8	8	30 (57 % of II)
	♂	4	9	10	23 (43 % of II)
Total		40	38	31	109

morbid state of the peripheral nerves This may be influenced by the *age and sex* of the patients. Tyler (29) claims that clinical neuropathy is more prevalent among males than females, and this trend is also apparent in other materials (5 30) However based on comparative studies of the motor conduction velocity Dobbelstein et al (5) questioned the presence of a sex difference. Tenckhoff et al (28) found a uniformly high age in their patients with neuropathy In other reports (13 29) clinical neuropathy has been observed in younger patients, too, but a systematic study of the influence of age in a larger material is not available.

The incidence of neurological symptoms and signs in 109 patients with varying degrees of renal failure is reported in two preceding papers (19 20) In an attempt to elucidate some of the above-mentioned problems the present study was designed to investigate the interaction of biochemical and biological variates in patients with and without signs of neuropathy An attempt has been made to establish a clinically useful quantitative measure for the clinical discrimination between these two groups by means of a multivariate statistical procedure.

MATERIAL AND METHODS

The 109 patients comprise 49 women and 60 men, from 13 to 73 years of age (mean 40 years). The criteria for selection and the clinical data, including neurological findings, have previously been presented (19 20). Fifty six patients presented clinical neuropathy defined as the presence of one ("mild") or more ("moderate to severe") clinical signs. Among the remaining 53 patients 12 had symptoms without signs. The isolated presence of cramps and/or restless legs was disregarded, since these symptoms were not considered part of the syndrome of uremic neuropathy (20).

The primary renal diseases, the age the kidney function, the degree and duration of azotemia, and the serum electrolytes of the two groups of patients are summarized Tables I, II and III respectively Table III also includes the mean values of the fasting blood sugar the serum B_{12} and folic acid concentrations measured in a limited number of unselected patients.

Conventional laboratory techniques were used. The endogenous creatinine clearance (C_c = ml/min) was determined as the mean of 3 consecutive 24-hour periods, and the duration of azotemia was defined as the time interval (months) from the earliest recorded serum creatinine level higher than 13 mg/l to the time of the examination.

Statistical procedures

Discrete variates. The comparison between two patient groups with respect to a discrete variate was based on a χ^2-test, with R 1 degrees of freedom. A 2 R contingency table was used. (R = the number of categories of the discrete variate.)

Continuous variates The statistical procedures applied in this study presume a normal (Gaussian) distribution of the variates. Consequently for each patient group, the empirical cumulative distribution of the single variates was plotted on normal and log normal probability paper Logarithmic transformation was performed when a variate showed a general tendency to follow a log normal distribution The *multivariate analysis* of two patient groups was performed according to the following principles. The equality of the two sets of mean values (the mean value vectors) was tested using Hotelling T^2 test or equivalently the F-test (12) When a significant (5%) difference was encountered, stepwise discriminant analysis was performed to permit a ranking of the variates according to their discriminating ability (3, 6). In stepwise discriminant analysis the single variate giving the best discrimination enters the analysis. At each step a test is made as to whether the discriminant function, using the variates already entered in the analysis, discriminates significantly less than the discriminant function of all variates (26).

RESULTS

Classification of patients. The frequencies of the primary renal diseases in patients with

Table II. *Age, kidney function, degree and duration of azotemia in patients with and without neuropathy*

The values are the antilogs of the mean values and 95 % confidence limits

	Sex	Clinical neuropathy Absent				Present				
			Mean	95 % limits	p		Mean	95 % limits	p	p
Age (y)	♀	30	41	11–70	N.S.	19	38	13–64	N.S.	N.S.
	♂	23	38	14–63		37	42	13–70		N.S.
Creatinine clearance (ml/min)	♀	30	8.8	0.5–145	N.S.	19	4.3	0.2–74	N.S.	$0.1 > p > 0.05$
	♂	23	13.1	1.5–109		37	5.0	0.6–40		< 0.01
Serum creatinine (mg/l)	♀	30	54	13–226	N.S.	19	74	19–270	$0.1 > p >$	N.S.
	♂	23	63	12–330		37	108	26–446	0.05	< 0.05
Serum urea (g/l)	♀	30	1.11	0.39–3.13	N.S.	19	1.48	0.50–4.39	N.S.	$0.1 > p > 0.05$
	♂	23	1.20	0.35–4.10		37	1.63	0.46–5.81		$0.1 > p > 0.05$
Duration of azotemia (mo.)	♀	26	25	2–270	N.S.	18	32	2–403	p 0.05	N.S.
	♂	23	17	1–270	N.S.	36	14	1–179		N.S.

p = p of differences between mean values in the two sexes. p = p of differences between mean values in patients with and without neuropathy. N.S. = $p > 0.10$

and without neuropathy (Table I) did not differ significantly either in women ($\chi^2_2 = 1.36$, $p > 0.10$) or in men ($\chi^2_2 = 1.87$, $p > 0.10$). The three diagnostic groups were therefore not distinguished in the following analysis.

A significant difference was encountered between the sex distribution in patients with and in those without neuropathy ($\chi^2 = 4.78$, $p < 0.05$) (Table I). Moreover, it was the clinical impression that females tended to be less severely affected. However, the percentage of females in the group of patients with "mild" neuropathy (46 %) did not differ significantly from that in patients with "moderate to severe" neuropathy (23 %) ($\chi^2 = 2.30$, $p > 0.10$). Due to the difference in the incidence of neuropathy the forthcoming analysis of numerical variates was conducted in each sex separately.

Table III. *Biochemical data in patients with chronic renal failure*

For explanation of symbols see Table II

	Sex	Clinical neuropathy Absent				Present				
			Mean	S.D.	p		Mean	S.D.	p	p
Sodium (mEq/l)	♀	30	138	3.8	N.S.	19	137	5.3	N.S.	N.S.
	♂	23	139	3.7		37	137	5.5		N.S.
Potassium (mEq/l)	♀	30	4.2	0.6	N.S.	19	3.8	0.9	p 0.01	N.S.
	♂	23	4.4	0.8		37	4.4	0.7		N.S.
Bicarbonate (mEq/l)	♀	30	21.6	3.2	N.S.	17	20.6	3.2	N.S.	N.S.
	♂	22	20.8	2.7		36	20.4	2.6		N.S.
Calcium (mg/100 ml)	♀	28	9.2	0.8	N.S.	15	9.0	0.7	N.S.	N.S.
	♂	22	9.2	0.5		34	8.8	1.2		N.S.
Phosphorus (mg/100 ml)	♀	28	5.7	2.5	N.S.	16	6.0	2.5	N.S.	N.S.
	♂	22	5.5	2.2		33	7.7	3.4		p 0.05
Magnesium (mEq/l)	♀	19	1.83	0.28	N.S.	14	2.16	0.34	N.S.	$p < 0.01$
	♂	17	1.99	0.27		31	2.06	0.50		N.S.
Vitamin B_{12} (pg/ml)	♀	6	522	212	N.S.	7	471	164	N.S.	N.S.
	♂	6	368	118		17	434	174		N.S.
Folic acid (µg/l)	♀	6	9.5	2.3	p 0.05	7	9.9	3.1	N.S.	N.S.
	♂	6	6.5	2.0		16	9.4	2.1		$p < 0.01$
Fasting blood sugar (mg/100 ml)	♀	20	75.7	18.5	N.S.	13	80.3	7.3	N.S.	N.S.
	♂	14	85.5	10.1		29	79.2	10.3		N.S.

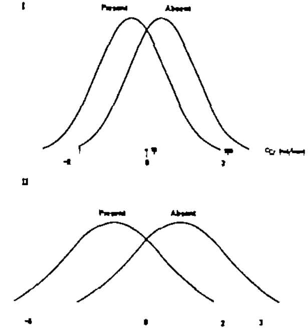


Fig. 1. The probability distributions of _ Male patients with neuropathy (left) and male patients without neuropathy (right) is used for the classification of new patients as follows: Patients in whom z assumes a value > 0 are classified in the group without neuropathy and patients in whom z assumes value < 0 are classified in the group with neuropathy. z is the estimate of theoretical linear function of the observations which minimizes the expected number of misclassifications when used according to the principles given above.

I. − 0.03421 log (creatinine clearance) 0.031

II. *0.05963 log (creatinine clearance) − 0.0015* age + 0.00531

III. 0.071 4 log (creatinine clearance) − 0.0019 age − 0.04046 log (se-creatinine) + 0.07428 log (urea) + 0.00185 se-N 0.00757 Se-K − 0.‥1083

Numerical variates. The best fit to the normal distribution was obtained by logarithmic ($\log_{10}$) transformation of the following variates: creatinine clearance, serum creatinine, serum urea, and the duration of azotemia. The other variates showed an approximately normal distribution on a linear scale.

It appears from Tables II and III that, in patients with neuropathy the duration of azotemia was significantly longer in females than in males ($p < 0.05$), while the mean serum potassium level was higher in males ($p < 0.01$). In other respects, in particular age, kidney function and degree of azotemia (Table II), the two sexes did not differ significantly

A complete set of data was available for the following six numerical variates: 1) creatinine clearance, 2) age, 3) serum creatinine, 4) serum urea, 5) serum sodium, and 6) serum potassium. These were included in the multivariate analysis.

In *females* the difference between the mean value vectors in patients with and without neuropathy was not significant ($F_{6,4} = 1.49$ $p > 0.05$) (the suffix in F indicates the degrees of freedom).

In *males* however a significant difference was present ($F_{6,3} = 4.49$ $p < 0.01$). In the stepwise discriminant analysis the creatinine clearance (log C_{Cr}) was *ranked as the single variate which gave* the best discrimination between the two groups of male patients ($F_{1,34} = 10.34$ $p < 0.01$) (Fig. 1 I). However the point of maximal discrimination ($C_{Cr} = 8.0$ ml/min, the intersection of the two normal curves in Fig. 1 I) misclassified ‥2 patients (37%) see Fig. 2, horizontal line. The discriminative power was significantly improved when all six variates were used (Fig. 1 III F $_{,31}$ = ‥95 $p < 0.025$) The age of the male patients was ranked next to the creatinine clearance in the order of discriminative effectiveness. *Using* both these variates in combination (Fig. 1 II), addition of the remaining four variates did not increase the discriminative effectiveness significantly (F $_{,31}$ = 1.08 $p > 0.10$)

In Fig. ‥ the creatinine clearance (log C) is plotted against the age of the male patients. *The line which gives the optimal discrimination* between patients with and without neuropathy was calculated from the discriminant function using these two variates alone (1). The line is the estimate of the theoretical line which gives the optimal discrimination provided that the incidences of patients with and without neuropathy are identical. However the intercept (but not the slope) of the line depends on the incidences of the two groups. These concepts were detailed by one of us in a previous paper (25). A neuropathy was *expected" in five of the ‥3 patients (2‥)* in whom clinical signs were missing, while the

presence of signs was "unexpected" in 12 of the 37 patients with neuropathy (35%). Thus 17 of all 60 males were misclassified (28%). It appears from the symbols used that 10 of the 17 misclassified patients belonged to the "symptomatic or mild" neuropathy groups.

The creatinine clearance is too complicated for routine laboratory control of patients with chronic renal failure. The stepwise discriminant analysis was therefore repeated, excluding the creatinine clearance. Serum creatinine was ranked as the single variate giving the best discrimination between males with and without neuropathy. The point of maximal discrimination (81 mg/l) misclassified 21 patients (35%), see Fig. 3 horizontal line. The discrimination based on the two highest ranking variates, the serum creatinine and age, did not differ significantly from that based on all variates ($p > 0.10$). Fig. 3 demonstrates the relationship between serum creatinine concentration

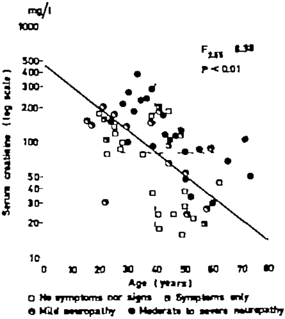


Fig. 3. The discrimination between male patients with and without neuropathy by log (serum creatinine) and age. Patients with observations above and below the line $= -0.07125 \cdot \log(\text{serum creatinine}) - 0.00136 \cdot \text{age} - 0.19024$ are classified in the group with and without neuropathy respectively. The dotted lines correspond to the line of discrimination when log (serum creatinine) (horizontal line) or age (vertical line) is used alone. The result of the F-test of the difference between the mean value vectors of log (serum creatinine) and age in the two populations is shown.

(log scale) and age. The line giving the optimal discrimination was calculated as above. A total of 15 patients were misclassified (25), eight of whom belonged to the intermediate groups, "symptomatic and mild" neuropathy. Hence the creatinine clearance can be replaced by the much simpler serum creatinine determination for the discrimination between males with and without neuropathy.

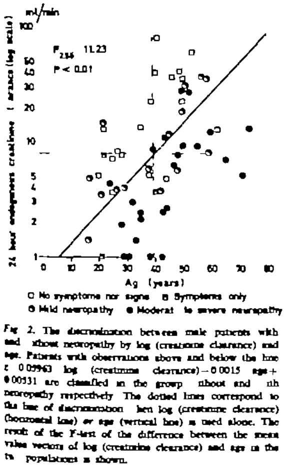


Fig. 2. The discrimination between male patients with and without neuropathy by log (creatinine clearance) and age. Patients with observations above and below the line $z = 0.05963 \cdot \log(\text{creatinine clearance}) - 0.0015 \cdot \text{age} + 0.00531$ are classified in the group without and with neuropathy respectively. The dotted lines correspond to the line of discrimination when log (creatinine clearance) (horizontal line) or age (vertical line) is used alone. The result of the F-test of the difference between the mean value vectors of log (creatinine clearance) and age in the two populations is shown.

DISCUSSION

The information obtained from a clinical study of the present type can only be evaluated in very general terms. The patient is far too complex as a pathophysiological "model" and the clinical examination is methodologically crude and unspecific. To alleviate part of these apparent disadvantages, proper statistical procedures may be a valuable remedy.

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MECHANICAL FRAGILITY OF ERYTHROCYTES IN NORMALS AND IN PATIENTS WITH HEART VALVE PROSTHESES

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Abstract. Hemolysis following insertion of heart ball valves shows great individual differences. Besides the mechanical properties of the prosthetic valves, individual variations in the mechanical fragility (MF) of the erythrocytes might be important in this respect. The MF has been studied with the hemoresistometer of Fleisch and Fleisch, which exposes the cells to a standardized trauma by rotation of the blood in a cylindrical chamber. The MF is defined as the amount of heme liberated. A normal material is presented, and the error of the method and the normal variation of the MF are estimated, using two different rotors. The hemoresistometer seems to be a valuable tool for evaluation of the MF offering several advantages compared to other methods. Reference has been made to a material of nine consecutive patients with heart valve disease examined before and after prosthetic ball valve implantation. All had a normal MF before the operation, and the mean MF remained normal in spite of moderate intravascular hemolysis developing in all but one patient postoperatively. In four patients with valve prostheses readmitted for hemolytic anemia the plasma heme concentrations were markedly increased, but the MF was normal. The question whether lowered MF may be the cause of the severe intravascular hemolysis developed by some patients remains unsettled, but the present study suggests that individual differences in MF are of minor importance for the development of severe hemolytic anemia in these patients.

Implantation of prosthetic ball valves in the heart provokes chronic intravascular hemolysis of varying degree in the majority of patients (2, 8, 9, 10, 11, 14, 16, 20). In about 5% hemolytic anemia develops (8, 9, 14). We studied patients with different types of aortic and mitral ball valve prostheses and found that the degree of hemolysis was largely determined by the type of valve implanted, indicating the direct mechanical trauma on the erythrocytes to be the main cause of hemolysis (8, 9). However there were great individual differences in red blood cell destruction within patient groups with the same type of valve. The question therefore arose whether the severity of hemolysis following heart valve replacement was due to individual differences in mechanical fragility (MF) of the erythrocytes.

Several methods have been used to measure the MF. Rotating tubes containing glass beads or other particles have been most commonly used (3, 7, 15, 17, 18). Andreasen (1) "atomized" suspensions of erythrocytes in order to avoid the influence of the plasma viscosity (12). In order to expose the cells to a standardized mechanical trauma, Fleisch and Fleisch (5) constructed the hemoresistometer in which heparinized blood is made to rotate in a cylindrical chamber.

In the present study the hemoresistometer has been used to determine the MF in normals, in patients before and after implantation of prosthetic ball valves, and in patients with hemolytic anemia following heart valve replacement. The accuracy of the method was also evaluated.

MATERIAL AND METHODS

Three groups of subjects were studied. Group I represented a normal material of 20 healthy volunteers and 47 patients with no blood or circulatory disorder. In group II were nine patients with valvular heart disease who were examined before and after replacement of the valves. Seven patients had Starr-Edwards aortic and two Starr-Edwards mitral valves inserted, in all but one (no. 4) the newer types with hollow Stellite (metallic) balls were used. Group III consisted of four patients who developed marked intravascular hemolysis with anemia following prosthetic valve implantation. Two patients (nos. 10 and 11) had Starr-Edwards aortic ball valves with hollow metal balls, one (no. 12) a Beall mitral prosthesis and the fourth patient (no. 13) had three valves replaced (Starr-Edwards aortic, Kay-Suzuki mitral and a Beall prosthesis in the tricuspid ostium).

The MF was determined by the use of the hemoresistometer (Fig. 1). Blood sampling was made as non-

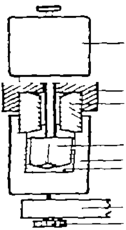


Fig. 1. Schematical drawing of the hemoresistometer after Fleisch and Fleisch. Ten millilitres of heparinized blood (*) is poured into the cylindrical container (.). The rotor *(4)* is screwed on to the motor spindle, the connecting part (3) is placed in position in the top piece *(2)*, and the container fastened by the arm (7) and the screw *(8)*. The motor *(1)* is started and turns the rotor at 3 000 rpm for 15 min.

traumatic as possible, in most cases during bed rest in the morning. Fifteen to twenty millilitres of blood were drawn into heparinized plastic syringes. Ten millilitres of the heparinized blood were transferred into the cylindrical container of the hemoresistometer, the equipment was put together and fastened. The motor was started and run for exactly 15 min at 3 000 rpm. The container was then disconnected, and ml of the blood was transferred into each of two plastic tubes. Two similar tubes were filled from the remainder of the heparinized blood in the syringe. The four tubes were then centrifugated at 900 G for 10 min, the plasma was carefully collected and transferred to four new tubes and centrifugated at _000 G for 30 min. The plasma was then analyzed for total heme pigments by the benzidine method of Crosby and *Furth* (4). The hematocrit was also determined.

The lactic dehydrogenase (LDH) was estimated in serum according to the method described by Wroblewski and laDue (19). The upper normal value in our laboratory is 200 μmol min l. The degree of intravascular hemolysis was predicted from the serum LDH level as described elsewhere (11).

RESULTS

The hemoresistometer was delivered with three different sets of rotors, designated "Hemolyse env 20 mg%" "H.e. 35 mg%" and "H.e. 60 mg%". We used the two rotors that provoked most hemolysis. The MF was defined as the amount of heme pigments (mg/100 ml) released during rotation of the whole blood in the hemoresistometer. *It was calculated as the difference* between plasma heme concentration after and before the fragility test. Duplicate determinations were always performed.

Table I shows the values in the normal individuals (group I). The heme concentration in the untreated plasma was somewhat higher than that found by others (4) who have proposed an upper normal limit of 5 mg/100 ml but it corresponded well with earlier results from our laboratory (13). The hemolysis provoked by the hemoresistometer was very slight, only about 0.2 and 0.5% of the total content of heme in the erythrocytes were detected in plasma after treatment of whole blood with the respective rotors. It is interesting to note that the scatter of the individual values was of the same magnitude for both rotors.

The error of the analysis of heme concentration was determined by duplicate estimations (Table II). As described, two plastic tubes were filled with heparin-blood directly from the syringe and blood rotated in the hemoresistometer was transferred to two other tubes. The difference in heme concentration between each of the tubes in a pair reflected the error in pipetting, centrifugation and analysis (Table III). Obviously the additional error due to pipetting and centrifugation was small. In calculating the MF the mean of paired observations was used, this reduced the error still further.

In order to obtain information about the total error of the method, including blood sampling,

Table I. *Plasma heme concentration and MF of erythrocytes in normals*

		Plasma heme pigments (mg/100 ml)					
		Before trauma		After trauma		MF	
Rotor type	No. of subjects	Mean	S.D.	Mean	S.D.	Mean	S.D.
H.e. 35 mg%	[illegible]	6.0	[illegible]	28.3	8.0	22.3	7.6
H.e. 60 mg%	9	5.9	2.5	67.8	7.4	61.9	6.3

treatment of the blood in the hemoresistometer and the heme analysis, blood was drawn from the same individual twice at an interval of about 30 min (Table IV). The spontaneous variation of the MF was evaluated by repeated examination of 17 subjects after 48 h.

The MF varies with the hematocrit values within wide limits (5) and therefore should be corrected to a chosen hematocrit value. This was not done in our material of normals: the hematocrit ranged from 38 to 46 with a mean of 42%.

Table II. *Errors of heme analysis. Duplicate determinations of plasma samples from different individuals*

	Plasma heme pigments (mg/100 ml)		
		After trauma with rotor	
	Before trauma	H.e. 35 mg	H 60 mg %
Mean of all determinations	5.9	27.5	68.4
Mean difference between duplicate determinations	0.75	2.3	4.4
S.D. of differences between duplicate determinations	1.15	3.2	5.4
No. of duplicate determinations	9	13	7

Table III. *Errors at centrifugation, pipetting and analysis. Differences between equally handled samples in a pair*

	Plasma heme pigments (mg/100 ml)		
		After trauma with rotor	
	Before trauma	H.e. 35 mg %	H 60 mg %
Mean of all determinations	6.0	27.9	67.0
Mean difference between determinations in pairs	1.35	3.5	5.8
S.D. of differences within pairs	2.0	4.3	8.0
No. of paired determinations	60	55	7

Table IV. *Error of the method and spontaneous variation. Differences in MF between blood samples obtained at intervals of about 30 min and 48 h, respectively. The rotor "H e 35 mg%" was used*

	MF of erythrocytes (blood drawn at interval)	
	30 min	48 h
Mean of all determinations	17.3	22.3
Mean difference between duplicate determinations	3.6	6.5
S.D. of difference between duplicate determinations	4.6	8.0
No. of duplicate determinations	7	17

Table V. *Plasma heme concentrations (mg/100 ml) and MF in group II before and after ball valve implantation*

A = untreated blood, B = blood treated in the hemoresistometer. The rotor "H e. 35 mg%" was used. The LDH values in serum after operations are listed

Pat. no.	Prosthesis	Before operation A	Before operation B	Before operation MF	After operation A	After operation B	After operation MF	LDH
1	Aortic	9.6	33.0	23.4	10.5	34.7	24.2	330
2	Aortic	8.1	26.7	18.6	7.2	14.7	7.5	353
3	Aortic	3.8	32.9	29.1	4.0	61.7	57.7	306
4	Mitral	4.6	33.8	29.2	10.3	40.0	29.7	210
5	Aortic	6.9	34.4	27.5	20.6	58.2	37.6	308
6	Mitral	5.7	24.7	19.0	5.5	22.0	16.5	340
7	Aortic	4.1	23.6	19.5	3.1	17.8	14.7	470
8	Aortic	6.2	37.5	31.3	2.8	18.3	15.5	380
9	Aortic	7.4	28.1	20.7	8.7	32.8	24.1	346
Mean		6.3	30.5	24.2	8.1	33.4	25.3	338
S.D		2.4	4.9	5.0				

Table V shows the MF in nine patients with valve disease before and 3–6 weeks after the prosthetic valve implantation (group II). The hematocrit level was normal in all patients both before and after the operation. Hemolysis was moderate in all cases, the erythrocyte destruction rates were between one and two times the normal as predicted from the serum LDH levels (11). The preoperative MF was within normal limits in all patients, while it was increased in one case (no. 3) after valve replacement. The plasma heme concentration was elevated in another operated patient (no. 5) but the MF was

Table VI. *Plasma heme concentrations before (A) and after treatment (B) in the hemoresistometer and MF corrected to a hematocrit value of 40 in group III*

Pat. no	Prosthesis	Heme pigments (mg/100 ml) A	B	MF corrected	Hb (g/100 ml)	Hct ( )	LDH (U/l)
10	Aortic	45.6	57.9	16.4	7.3	30	1 570
11	Aortic	65.2	89.6	27.4	9.1	32	1 160
1	Mitral	39.1	71.0	35.0	10.1	35	920
13	Triple	25.5	38.7	15.8	10.4	34	765

normal. There was no correlation between the MF and the degree of hemolysis postoperatively.

In group III, operated patients readmitted because of severe hemolysis, the hematocrit values were low. The MF was therefore corrected to a hematocrit value of 40 (Table VI). The plasma heme concentration was markedly elevated in all four patients, while the MF was found to be normal.

DISCUSSION

The great individual differences in hemolysis following insertion of heart valve prostheses (8, 9) could be due to individual differences in the red blood cell resistance to mechanical trauma. This factor has not previously been studied in such patients, probably because most routine methods for MF determination are rather rough and difficult to standardize. Because the expected differences in MF are small, a strictly standardized and sensitive technique is a matter of necessity. The hemoresistometer is claimed to have several advantages compared to the common methods using rotating test tubes containing glass beads (5). The trauma is homogenous and is provoked by rapid rotation of the blood instead of crushing the erythrocytes by beads. Ideally, only fragile erythrocytes should be destroyed. The hemoresistometer provokes a relatively low degree of hemolysis; its traumatic effect is more similar to the physiological trauma of the erythrocytes in the circulation. Because the test chamber is built of plexiglass, the contact with air minimal, and the duration of the test short, chemical influence on the erythrocyte membrane is probably negligible.

We mainly used the rotor designated "Hemolyse env. 35 mg%" because we wanted a direct comparison of our normal material with the findings of Fleisch and Fleisch (5). We found an approximately similar mean MF and a somewhat smaller normal variation (Table I). The error of the various steps of the procedure was analyzed and the method was found to be sensitive and the results reproducible. The differences observed represented very small variations in the degree of hemolysis. We believe that the rotor producing most hemolysis should be preferred. The hemolysis provoked by this rotor is far less than by other methods (1, 3, 7, 18) and probably lies within "physiological" limits. Using this rotor one may be able to detect decreased as well as increased MF.

In most other methods (3, 7, 18) the normal variation of the MF (in per cent of the mean value) has been as great or greater than that found with the hemoresistometer, and the erythrocyte destruction higher. Andreasen (1) used a quite different technique, atomizing a suspension of erythrocytes. He found a much smaller variation of the MF in normals, probably because the influence of plasma viscosity was eliminated.

Preoperatively, patients with valvular heart disease showed a normal MF of the erythrocytes. However, no significant changes occurred in the MF after ball valve insertion. Eight patients developed moderate intravascular hemolysis, but there was no correlation between the preoperative MF and the degree of hemolysis postoperatively. Thus the individual differences in hemolysis following prosthetic valve implantation could not be explained by different MF of the erythrocytes. However, we would need preoperative estimations of the MF in patients who later develop severe hemolysis in order definitely to settle the question of the influence of individual differences in the MF.

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INFLUENCE OF NICOTINIC ACID ON INDIVIDUAL PLASMA PHOSPHOLIPIDS IN HYPERCHOLESTEROLEMIC SUBJECTS

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Abstract. In ten patients with hypercholesterolemia plasma lipids, including individual phospholipids, were analysed before and 2 to 18 months after high-dose nicotinic acid therapy. Free fatty acids were increased, while cholesterol, triglycerides and total phospholipids decreased. Among the individual phospholipids there was a significant decrease of lysolecithin in absolute as well as relative amounts. The other phospholipid fractions did not change significantly. The possible mechanism of nicotinic acid effects on plasma lipids are discussed.

Increased plasma lipid levels are correlated with the development of ischemic heart disease. In the hope of effecting prophylaxis against the development or progress of atherosclerosis, attempts are often made to lower plasma lipids by various means. Diets have been extensively used, but also drug therapy often with success. One of the most prescribed drugs has been nicotinic acid, introduced for this purpose by Altschul et al. (1). The mechanism of its actions is not known in detail.

As lipid-lowering therapy must extend over long periods, it is of interest to study their effect in as much detail as possible. The effect of long term nicotinic acid on several lipid fractions has been repeatedly reported, but studies on plasma phospholipids have only been concerned with the total amounts. We thus decided to study the effects of nicotinic acid on individual plasma phospholipids.

MATERIAL AND METHODS

The material consisted of 10 patients, 4 men and 6 women. Their mean age was 59 years (range 43–71). Nine of the patients had angina pectoris and some had had myocardial infarction in the past, but not recently. One patient had been examined because of xanthelasmata. None of the patients was diabetic (glucose tolerance was, however, not investigated). Most of the patients were taking some drugs for their cardiovascular disease, e.g. digitalis, nitroglycerin or dicoumarol. A rigid diet was not prescribed.

Plasma lipids were examined before institution of therapy with nicotinic acid in the form of Nicangin® (Draco, Lund, Sweden), which is a buffered preparation, soluble in water. The initial dosage was 0.25 g t.i.d., which was gradually increased up to 1 g t.i.d., except for one patient who could not tolerate more than 0.5 g t.i.d. on account of subjective side-effects in the form of flushing, which did not subside with time. Otherwise the medication was well tolerated. Liver function tests were not routinely performed, but none of the patients had pruritus or evidence of jaundice.

Examination of plasma lipids was repeated after nicotinic acid had been taken for 2–18 months (mean 8.5).

Heparinized plasma was obtained in the fasting state, and free fatty acids, total and free cholesterol, triglycerides and total and individual phospholipids were analyzed as previously described (4). Analyses were repeated on the same plasma after incubation at 37°C for 6 h.

For statistical analysis the directions of Snedecor (27) were followed. For judgment of changes during therapy the paired *t*-test was used.

RESULTS

Mean values and standard errors of the means before and during treatment with nicotinic acid are presented in Table I. Individual phospholipids are given both as the percentage of total phospholipids and as absolute values.

Free fatty acids were significantly higher during treatment. The reduction of triglycerides and of total cholesterol is also significant. Although the reduction of free cholesterol is relatively the same as for total cholesterol, it does not reach statistical significance in this material.

Table I. *Plasma lipids in 10 subjects before and during therapy with nicotinic acid*

FFA = free fatty acids, TC = total cholesterol, FC = free cholesterol, TG = triglycerides, PL = phospholipids, PE = phosphatidylethanolamine, Lec = lecithin, Sph = sphingomyelin, LL = lysolecithin, S.E.M. = standard error of the mean

	FFA	TC	FC	TG	PL	of total PL				mM/l			
	(mEq/l)	(mg/dl)	(mg/dl)	(mM/l)	(mM/l)	PE	Lec	Sph	LL	PE	Lec	Sph	LL
Before therapy													
Mean	0.80	318	91	1.42	3.80	1.4	69.2	24.0	5.4	0.06	2.62	0.91	0.20
S.E.M.	0.05	16.0	5.7	0.07	0.13	0.15	0.78	0.78	0.45	0.007	0.08	0.03	0.017
During therapy													
Mean	1.16	254	78	0.94	3.56	2.0	69.7	23.8	4.5	0.07	2.48	0.85	0.16
S.E.M.	0.15	20.8	5.4	0.10	0.16	0.28	0.50	0.54	0.42	0.012	0.10	0.05	0.013
Difference													
Mean	−0.36	−64	−14	−0.50	−0.24	+0.6	−0.5	−0.2	−0.9	+0.02	0.15	−0.06	−0.05
S.E.M.	0.12	18.0	6.5	0.11	0.10	0.34	0.77	0.79	0.37	0.014	0.07	0.04	0.012
p	<0.05	<0.01	ns	<0.01	<0.05	ns	ns	ns	<0.05	ns	ns	ns	<0.01

Total phospholipids were significantly lower during treatment. Individual phospholipids were also decreased, except phosphatidylethanolamine. However only in the case of lysolecithin is the decrease significant, both in absolute amounts and as percentage of total phospholipids.

After incubation of normal plasma an increase of free fatty acids and of lysolecithin was found, while triglycerides and lecithin were decreased (4). There was no significant difference in this respect before and during therapy with nicotinic acid. The results are presented in Table II.

DISCUSSION

Since the work of Altschul et al. (1) nicotinic acid in large doses has been used extensively because of its lipid-lowering capacity. A large number of reports have appeared on plasma or serum lipid levels during such treatment, and our results are partly in accord with the findings in the literature (3, 9, 23).

While nicotinic acid inhibits lipolysis (6) and thus in acute experiments lowers free fatty acids, it has repeatedly been found that in extended studies there is a rebound phenomenon, so that the fasting free fatty acids are actually increased (10, 12, 24). This is in accord with our findings, as is also the decrease of triglycerides and total cholesterol (3, 9, 23).

Total phospholipids have sometimes been measured in long-term studies and a slight reduction has usually been noted (3, 9). However, as far as we know, no previous study has been published on the levels of individual phospholipids.

Our findings indicate that nicotinic acid has only a slight influence on the composition of the phospholipid fractions. The only significant change was the decrease of lysolecithin both as absolute values and as percentage of total phospholipids.

The mechanisms by which nicotinic acid lowers plasma lipids are not established. It has been found, for instance, that nicotinic acid may decrease biosynthesis of cholesterol (14) or increase its oxidation (19). Inhibition of lipolysis has also been considered to be of importance. Carlson et al. (11) have suggested that this inhibition leads primarily to a decrease of very-low-density lipoproteins (VLDL) and secondarily to a decrease of low-density lipoproteins (LDL). Several other possible mechanisms have also been discussed (23).

Table II. *Changes in plasma lipid and phospholipid values after incubation (6 h, 37°C)*

Symbols as in Table I. None of the differences is significant

	FFA (mEq/l)	FC (mM/l)	TG (mM/l)	Lec (mM/l)	LL (mM/l)
Before nicotinic acid (A)					
Mean	+0.21	−0.15	−0.11	−0.18	−0.17
S.E.M.	0.03	0.104	0.036	0.029	0.014
After nicotinic acid (B)					
Mean	+0.16	−0.28	−0.06	−0.11	−0.15
S.E.M.	0.03	0.074	0.017	0.017	0.012
Difference (B − A)					
Mean	−0.05	−0.13	+0.06	+0.07	−0.03
S.E.M.	0.04	0.12	0.032	0.032	0.014

Our patients with normal triglyceride levels must have had minor amounts of VLDL and the lowered levels of cholesterol must have been due mainly to a reduction of LDL. The individual phospholipid composition in various lipoproteins differs (3). In the LDL (or β-lipoproteins) there is relatively more sphingomyelin and relatively less lecithin than in whole plasma. A reduction of the LDL fraction would thus tend to reduce the relative percentage of sphingomyelin in plasma and elevate that of lecithin. This was not found in the present study which may be explained by a too small reduction of the LDL fraction and the restricted number of persons studied.

Previous studies have shown that lysolecithin is mainly found in the high-density lipoproteins (which on electrophoresis correspond to the α-lipoproteins) or even in the so-called very high-density lipoproteins (density > 1.21) (25 30). The reduction of lysolecithin in absolute as well as relative amount after nicotinic acid therapy suggests that this drug may influence these heavy lipoprotein fractions. The decrease of lysolecithin cannot depend only on a reduction of LDL, as in hypercholesterolemia (which is due to an increase of LDL) the absolute amount of lysolecithin was normal compared to a control material and reduced when expressed as percentage of total phospholipids (37). An isolated decrease of LDL would thus tend to increase the percentage of lysolecithin, which was not the case in the present study

Lowered levels of lysolecithin have previously been found in patients with liver disease (15 20, 26), in pregnancy (28 31) after administration of estrogen (29) and some contraceptive preparations (6, 7 21). Older men had lower levels than younger while there was no age difference in women (4).

Nicotinic acid is known to affect the liver sometimes leading to severe liver damage (2, 18), and it is possible that our findings may indicate a subclinical liver affection. However the studies on individual phospholipids in liver disease have been concerned with rather severely affected patients and it cannot be concluded that minor liver injury induces alterations in the plasma phospholipid composition.

The lowering of lysolecithin in pregnancy and after certain contraceptive preparations is probably due to an estrogen effect, possibly mediated via the liver As far as we know it has not been shown that nicotinic acid has any estrogenic effects, although Hirschhorn & Seegers (17) mentioned estrogenic" side-effects in the form of menstrual irregularities during nicotinic acid therapy Further investigations in this direction may be indicated, as estrogens have a well-known lipid-lowering effect (13 22).

The lysolecithin in plasma seems mainly to be produced from lecithin via the acyl-transferase mechanism described by Glomset (16). Possibly also phospholipase A may be of importance (5). Our findings, that changes in plasma lipids during incubation were normal and similar before and during nicotinic acid therapy indicate that these enzymes are not affected by the drug. However our method is only a rough measure of enzyme activities and a slight effect is by no means excluded.

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SOME BIOLOGICAL PROPERTIES OF PURIFIED SULFATION FACTOR (SF) FROM HUMAN PLASMA

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Abstract. The sulfation factor (SF) was purified from human plasma. This factor also stimulates the uptake and conversion of glucose-1-^{14}C to $^{14}CO_2$ in rat epididymal fat tissue after addition of insulin antibodies to the incubation medium (so-called nonsuppressible insulin-like activity ILA). During purification of plasma SF it was not possible to separate nonsuppressible ILA from SF activity: one unit of SF corresponding to about 200 U of nonsuppressible ILA. This finding indicates that SF activity and nonsuppressible ILA are inherent in the same molecule. Human growth hormone was shown to induce the occurrence of SF activity in a crude microsomal fraction from rat liver.

Pituitary growth hormone (GH) in vivo exerts various metabolic actions on cartilage, skeletal muscle, heart, liver and adipose tissue (8, 14, 22, 23, 36). Stimulation of amino acid transport and of protein synthesis in cartilage and diaphragm have been observed (9, 10, 25, 34, 35). On the other hand, GH in vitro has scarcely any effect on the incorporation of labelled sulfate and amino acids into cartilage from hypophysectomized rats (16, 33). The difference between the in vivo and the in vitro effects of GH on cartilage was found to be due to the fact that the hormone in vivo induced the occurrence of a serum component that stimulated sulfate uptake, the so-called sulfation factor (SF) (33).

In the present study we have investigated the possibility that SF may also mediate other effects of GH. For this reason, the effect of purified plasma SF was tested on the conversion of glucose to CO_2 in adipose tissue. Since the production site of SF is unknown we also studied the occurrence of SF in a crude microsomal fraction from rat liver during incubation with human growth hormone (HGH).

MATERIAL AND METHODS

Purification of sulfation factor from human plasma

Fifty liters of outdated, frozen, human plasma from blood donors were pooled. In the subsequent purification procedure and biological assays the term "plasma equivalents" (pl eqv.) is used to indicate the original volume of plasma in ml, from which each preparation was derived. The purification steps used in the present study are shown in Fig. 1.

The plasma was lyophilized, suspended, and homogenized at +4°C in 0.9% w/v NaCl to 1/4 of the original plasma volume. To 16 volumes of this suspension a mixture of 48 volumes of 96% w/v ethanol and 1 volume of conc. HCl was added at +4°C while stirring. After centrifugation the supernatant was brought to pH 8.5 by 1 N NaOH and centrifuged. The supernatant was cooled to −16°C and 4 volumes of prechilled acetone-ethanol (5:3) were added. After 24 hours at −16°C the supernatant was decanted and the precipitate lyophilized (the acid ethanol extract).

The acid ethanol extract was dissolved in 0.2 N NH_4 acetate, pH 5.6, and passed through a Dowex 50 W X2 (H^+) column prewashed with 0.2 N NH_4 acetate, pH 5.6. The column was subsequently washed with 0.2 N NH_4 acetate, pH 5.6, 1.6 N CH_3COOH, pH 2.0, aqua dest., 0.1 M diethylamine and 0.2 N NH_4OH. The eluates were lyophilized.

The diethylamine eluate was applied to a column of Sephadex G-25 (fine) equilibrated with 1 M or 0.1 M CH_3COOH containing 0.01 M mercaptoethanol. The column was calibrated with blue dextran (2·10^6 average mol.wt.), glucagon (mol.wt. 3 483), cyanocobalamine (B_{12}) (mol.wt. 1 356) and acetone (mol.wt. 58). After determination of absorbance at 260 nm the individual fractions were pooled to form larger fractions, and lyophilized.

The lyophilized Sephadex fractions, which showed biological activity (see below) were dissolved in a minimal amount of pyridine-acetate buffer pH 6.4 and applied as a band to a sheet of Whatman 3 MM paper with about 1 mg of dried sample/cm. High voltage electrophoresis was then carried out in pyridine–acetate buffer, pH 6.4, at 60 V/cm for 50 min. A guide strip of the dried paper was cut and stained with sulphydrile-cadmium acetate. All ninhydrin-positive bands were cut from the paper eluted with

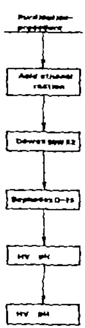


Fig. 1 Purification scheme for sulfation factor from human plasma. *A*, pooled human plasma. *B* dried acetone–ethanol precipitate. *C* lyophilized diethylamine eluate from *Dowex 50 W X2*. *D* low molecular region from Sephadex G-25 *E*, the neutral band from paper electrophoresis pH 6.5

0.1 N CH_3COOH and lyophilized. The biologically active fractions were applied to the origin of another Whatman 3 MM paper and electrophoresis carried out in acetate formate buffer pH 4.0, at 60 V/cm for 60 min. All ninhydrin-positive bands were eluted, and those used for bioassay were lyophilized together with human albumin.

Preparation of sulfation factor activity from rat liver microsomes

Normal Sprague Dawley male rats (from AB Anticimex, Norrviken, Sweden) were kept for 10 days in our laboratory with free access to food and drinking water. At the time of the experiments their body weights were 180–220 g. The preparation of tissue samples was carried out at 0–3°C. The rats were killed by a blow on the head. The livers were rapidly removed, weighed, and homogenized for 90 sec in a Potter Elvehjem type of glass homogenizer (Teflon pestle) with 2 volumes of ice-cold 0.25 M sucrose solution. A liver supernatant fraction, free from unbroken cells, nuclei, and mitochondria was prepared by centrifugation of the homogenate at 9 000 g for 20 min in a Sorvall centrifuge: this supernatant fraction containing microsomes was used in the incubation study.

The incubations were carried out at 37°C in a three-necked round-bottomed flask. To 20 ml of the supernatant containing the liver microsomes from 6.6 g of liver were added 15 ml cofactor solution containing 27 µmoles NADH, 300 µmoles glucose-6-phosphate, 40 µmoles nicotinamide and 300 µmoles $MgSO_4$. Finally 15 ml 0.2 M Tris-HCl, pH 7.4 and 0.09 M NaCl containing 30 mg of monomeric HGH, were added. The mixture was slowly stirred through a glass cooler to avoid evaporation. A mixture of 95% O_2 and 5% CO_2 was bubbled through the solution during the incubation period. Samples (2.0 ml) were taken at 0, 10, 20 and 30 min and 1, 2, 3, 4 and 6 h of incubation and immediately mixed with an equal volume of ice-cold ethanol. After centrifugation the supernatant was diluted and lyophilized in the presence of human albumin before the bioassay.

The HGH (Kabi Pharmaceutical House, Stockholm, Sweden) was monomeric and prepared according to the method of Roos et al. (32). One mg of this HGH corresponded to 2.0 IU of the *first International reference* preparation for HGH by immunoassay (National Institute for Medical Research, London). The insulin used was crystalline porcine insulin containing 24 IU/mg (N.I.L., Gentofte, Denmark). The human albumin purified according to Cohn et al. (7) was further purified. After 60 min pretreatment with 0.2 M 2-mercaptoethanol the albumin was separated over Sephadex G-25 equilibrated with 0.1 M CH_3COOH containing 0.01 M mercaptoethanol (3).

Bioassay of sulfation factor (SF) activity

The in vitro incorporation of labelled sulfate into cartilage from embryonic chickens was determined as described earlier by Hall (16). The different fractions were always lyophilized before the addition of the incubation medium and were assumed to contain no sulfate. Purified human albumin in a conc. of 0.5–1 mg/ml was added to the medium when purified SF or porcine insulin were used in the assay to avoid losses due to absorption by glassware. An arbitrary reference serum from a healthy male was used. One unit of SF activity is here defined as the biological activity in 1 ml of this reference serum. In the quantitative assays, 4-point assays with one-way analysis of variance were used and in single determinations 3-point assays.

Bioassay of insulin-like activity (ILA) and nonsuppressible ILA

Epididymal adipose tissue was used from 6 male rats, fasted for 120 hours and thereafter fed for 24 hours. The epididymal adipose tissue from each rat was divided into six equal pieces and incubated separately for 1 hour in Krebs-Ringer bicarbonate buffer containing glucose-1-^{14}C, 200 mg of glucose and 200 mg of albumin/100 ml. The conversion of glucose-1-^{14}C into $^{14}CO_2$ was measured (31). In the bioassays of nonsuppressible ILA the fractions were preincubated for 1 hour with insulin antibodies (concentration 1/10 000). This amount of antibodies totally inhibits the effect of 500 µU porcine insulin/ml on the adipose tissue. As reference porcine insulin in concentrations of 200 µU, 50 µU and 12.5 µU/ml, respectively were used. Two or three doses of the tested sample were employed. At each dose level 6 different rats were used. Variances were determined by two-way analysis of variance and in the quantitative estimations 4-point assays were applied. The results were statistically analyzed according to Finney (12) with a computer (PDP8).

Immunoassay of insulin

Insulin content of plasma and plasma fractions were determined by radioimmunoassay according to Hales and Randle (15).

Protein determination

Protein measurements were made by the method of ninhydrin determination after alkaline hydrolysis (20). Purified human albumin was used as standard.

RESULTS

1 Influence of insulin on the ^{35}S uptake by embryonic chick cartilage

Porcine insulin was tested at concentrations between 62.5 and 4 000 μU/ml, which induced a slight, but not always significant, stimulation of the uptake of labelled sulfate by the cartilagenous pelvises from embryonic chickens (Fig. 2). After reduction of insulin with 2 mercaptoethanol, both its radioimmunological properties and its effect on cartilage were absent.

2 SF activity, ILA, nonsuppressible ILA and radioimmunoactive insulin (IRI) in original plasma, acid ethanol extract and the eluates after ion exchange chromatography

The pooled plasma, used for purification of SF contained 0.57 U of SF and 22 μU of IRI/ml. The content of nonsuppressible ILA was 127 μU/ml and of ILA 225 μU/ml. Hitherto the acid ethanol extraction of plasma has been performed several times, and up to 40% of SF activity can be recovered. In the present study only 18% of the SF activity of the original plasma was recovered in the acid ethanol extract. The nonsuppressible ILA and the ILA recovered were 21 and 43% respectively. The acid ethanol extract contained 0.5 SF units and 0.13 nonsuppressible ILA mU/mg protein. The recovery of IRI was 35%.

When the acid ethanol extract (AE) was applied to Dowex 50 W X2 (H) in 0.2 M NH_4 acetate, pH 5.6, about 80% of the applied SF activity and about 40% of the applied nonsuppressible ILA was recovered in the diethylamine eluate. About 80% of IRI was also recovered in the same eluate.

Reduction with 0.1 M 2-mercaptoethanol, pH 8.5 and thereafter exposed freely to air totally destroyed the radioimmunologic and biologic in vitro action of insulin, whereas SF activity and nonsuppressible ILA remained unchanged.

3 SF activity and nonsuppressible ILA during gel chromatography and high-voltage electrophoresis

The diethylamine eluate of the AE extract, treated with -mercaptoethanol was further purified by

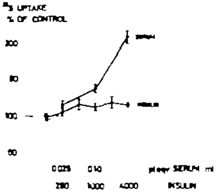


Fig. 2. The in vitro effect of porcine insulin on the uptake of labelled sulfate by embryonic chick cartilage compared with the effect of normal human serum. Each point represents the mean of 6 cartilages. Range bars indicate the standard error of the mean. The uptake in the basal medium containing amino acids — — and after addition of albumin – – –. Pl. eqv. is used to indicate the original volume of plasma in ml.

gel chromatography. Fig. 3 shows the results after using Sephadex G-25 equilibrated with 1 M CH_3COOH containing 0.01 M mercaptoethanol. SF activity occurred over a wide range corresponding to a low molecular weight. The recovery of SF and ILA activities was 100% after gel chromatography. These fractions contained about 70 U SF activity and 20 mU nonsuppressible ILA/mg protein. The total recovery of SF activity and nonsuppressible ILA from plasma were 15 and 19% respectively.

The pooled, biologically active Sephadex fractions were subjected to high-voltage paper electrophoresis at pH 6.4. Both SF activity and nonsuppressible ILA were observed only in the neutral band. This band was eluated and lyophilized together with purified human albumin and, thereafter subjected to high-voltage paper electrophoresis at pH 2.0. There was further resolution of the neutral band into seven ninhydrin-positive bands. Again both SF activity and nonsuppressible ILA appeared in the same band with a mobility between methionine and glycine.

4 Recovery of SF activity and nonsuppressible ILA during purification of SF

Table I shows the amount of SF activity and nonsuppressible ILA at each step of purification. A similar amount of SF activity and nonsuppressible

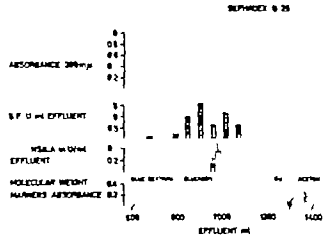


Fig. 3. Ascending gel chromatography on Sephadex G-25 (fine) equilibrated with 1 M CH_3COOH containing *0.01 M* 2-mercaptoethanol. The lyophilized diethylamine eluate from 4 400 pl.eqv. AE extract was applied to the column (5 × 86 cm) in volume of 10 ml. After measuring the absorbance at 260 nm, the fractions were pooled as the vertical lines indicate and assayed for biological activity. SF activity (hatched columns) is shown in U/ml effluent. ILA (hatched column) and nonsuppressible ILA (open column) are shown in mU/ml effluent with their fiducial limits ($p = 0.95$).

ILA was recovered. At each step the nonsuppressible ILA was 100–300 μU/U SF activity.

5. Induction of SF activity in rat liver microsomes

The lyophilized samples taken during the incubation period were tested for SF activity. The sample used as control was taken at the start of the incubation period immediately upon addition of HGH. No SF activity occurred during the first 20 min and a plateau was obtained between 30 min and 4 hours of incubation. The amount of the supernatant tested per ml incubation medium in Fig. 4 was derived from 17 mg rat liver. As can be seen from Fig. 4 this dose causes 100% increase above the control at 2 and 3 hours of incubation. We were unable to increase this stimulation further by increasing the amount of liver supernatant in the assay (probably due to the presence of inhibitors).

The biologically active fractions after 1, 2 and 3 hours of incubation were pooled and lyophilized in the presence of human albumin and then subjected to gel chromatography on a Sephadex G-25 column (2.5 × 45 cm) equilibrated with 0.1 M CH_3COOH. Biological activity was then recovered in the same region as the partial purified SF from human plasma.

DISCUSSION

It is well established that serum from rats and from humans possesses sulfation factor (SF) activity and that this activity occurs under the influence of growth hormone (GH) and human growth hormone (HGH) (2, 11, 16, 17, 33). The sulfation of chondroitin sulfate in cartilage, induced by serum containing SF activity, has been shown to occur after the stimulation of protein

Table I. *Sulfation factor (SF) activity, nonsuppressible insulin-like activity (NSILA) and radioimmunological insulin (IRI) during purification of 10 000 ml human plasma. The biological activity is given with the fiducial limits ($p = 0.95$)*

Preparation	SF (U)	NSILA (mU)	IRI (mU)	NSILA (μU)/SF (U)
Plasma	5 650 (3 000–9 110)	1 270 (710–2 780)	220	225
AE extract	1 040 (680–1 490)	265 (33–609)	77	255
Diethylamine eluate from Dowex 50 W X2	860 (410–1 400)	98 (7–240)	59	115
Low mol. region from Sephadex G-25	840 (440–1 290)	239 (96–428)	0	285
Neutral band from HVE pH 6.5	430 (160–1 270)	60 (16–98)	0	140
HVE pH 2.0	104 (39–179)	33 (1–113)	0	310



Fig. 4. SF activity in the supernatants from the incubation of liver microsomes and HGH taken at various intervals. The bars represent the mean (6 cartilages) and the range bars the standard error of the mean.

synthesis in this tissue (1, 9, 10). The close connection between sulfation and protein synthesis has been further illustrated (34, 35). During purification of SF from serum of patients with acromegaly it was not possible to separate its effect on the incorporation of sulfate and thymidine into cartilage from hypophysectomized rats (39, 40).

Thus apparently the effect of HGH on protein synthesis might be mediated through SF. In this connection it is of interest to note that normal serum, serum fractions containing SF activity and pure insulin stimulate the incorporation of amino acids into proteins of diaphragms from hypophysectomized rats (34, 35). Furthermore, it may be mentioned, that studies in our laboratories have demonstrated that purified SF, containing no insulin, had an enhancing effect on the transport of the synthetic amino acid α-amino-isobuturic acid (AIB) into diaphragms of hypophysectomized rats (38).

The close connection between GH in vivo and insulin with regard to protein synthesis is well established. Since this activity of HGH might be mediated by SF it was thought expedient to investigate the possibility of a connection between SF and the so-called insulin-like activity in plasma (ILA). Only a few studies in the literature deal with the role of GH on ILA in plasma. Randle and Young (30) showed that ILA decreased to undetectable values in the rat, following hypophysectomy and that treatment with GH prevented this decrease. In man decreased ILA in serum was demonstrated in hypopituitary patients and increased ILA in acromegaly (24, 28, 29, 41). Furthermore, ILA in serum was observed to have increased 4 hours after intravenous injection of HGH (42). In this respect its behavior resembles that of SF activity (17).

Our studies were directed towards the possible connection between SF and the remaining ILA in serum (about 90%) after suppression of insulin with antibodies, the so-called nonsuppressible ILA (13). In the procedure applied by us to isolate SF from plasma, the acid ethanol extraction is the first step. This extract is previously known to contain both insulin and nonsuppressible ILA (5, 6, 21, 27), and has previously been shown to contain also SF activity (39). We recovered about 20% of the original nonsuppressible ILA in human plasma, which corresponds to that found by other authors (27). On the other hand nonsuppressible ILA prepared from a Cohn fraction of plasma or acetone powder of plasma only represents 2.5–5% of the original nonsuppressible ILA in plasma (5, 6, 21). During further purification of SF from plasma of patients with acromegaly similarities regarding both molecular size and pK-values between SF and nonsuppressible ILA of low molecular size have been observed (21, 40). These findings indicate that SF might be identical with nonsuppressible ILA.

The present investigation has shown that it was impossible to separate the SF activity on cartilage from the nonsuppressible insulin-like activity on adipose tissue. The recovery of SF activity and nonsuppressible ILA during purification from normal human plasma was very similar. At each purification step there was about 200 μU of nonsuppressible ILA/U SF. At present it cannot be definitely stated that our purified SF is a homogeneous compound. Therefore, it cannot be totally excluded that SF and nonsuppressible insulin

Congress Announcements

The 25th-anniversary International Continuation Course for Physicians will be held in Karlsbad, Czechoslovakia, September 27 to October 1 1971

Secretariat

Dr J Benda, CSc. Research Institute of Balneology Pavlovova 1 Karlovy Vary Karlsbad, Czechoslovakia.

The 57th Annual Clinical Congress of the American College of Surgeons will be held in Atlantic City New Jersey USA, October 18 to 22, 1971

Information on official forms for registration, housing and postgraduate courses available after June 1 Contact: Mr T E McGinnis, American College of Surgeons, 55 East Erie Street, Chicago, Ill. 60611 USA.

IFAC Symposium on Automatic Control and Computers in the Mechanical Field will be held at the Palais des Congrès in Brussels, Belgium, September 27 to October 1 1971

Organizer

The Institut Belge de Régulation et d'Automatisme (I.B.R.A.)

Registration fees

Up to Aug. 31 B.F 2 500: — from Sept. 1 B.F 3 000· — Payments are to be made to the accounts of the Institut Belge de Régulation et d'Automatisme, Bruxelles, no 662 93 at the Office des Chèques Postaux, Bruxelles, or no. 64 026 at the Société Générale de Banque, Bruxelles. The fee includes the supply of the preprints.

Information

DZZZZ Secrétariat de l' I.B.R.A Rue Ravenstein 3, B-1000, Brussels, Belgium.

Acta med. scand. Vol. 190 pp. 145–148, 1971

PROTECTION OF BLOOD CELLS BY PLASMA PROTEINS

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Abstract. A study has been made as to whether plasma proteins might interfere with the behavior of blood cells. In media deprived of plasma, erythrocytes and leukocytes became irregularly shaped, tended to aggregate, to show poor recovery after centrifugation and to become stained by supravital dyes. A trace of plasma or serum was sufficient to protect the cells. Similar results were obtained with plasma proteins, of which fibrinogen was more effective than albumin or gammaglobulin. It is suggested that plasma proteins protect the cell membrane.

Pappenheimer noticed more than 50 years ago (6) that serum "even in dilute amount" protected cells from being stained, this has later been verified (1). The beneficial influence of serum or plasma in cell cultivation is well established, though it is not known which part of serum or plasma is essential.

In working with problems concerning the vitality of leukocytes, the author noticed that the presence of plasma greatly interfered with the results. It was therefore decided to study the possible influence of plasma proteins on blood corpuscles.

MATERIAL

The separation techniques of blood cells have been described elsewhere (10). The erythrocytes and platelets obtained were practically pure, while the polymorphonuclear leukocytes were slightly contaminated with erythrocytes and platelets (10).

The cell-suspending media used were Ringer phosphate glucose (12), Earle's solution (Oxoid Ltd., England), M 199 prepared from Parker's solution (Orion, Finland) and Hanks' solution (Difco, USA).

The dyes used in supravital staining were trypan (Merck, Germany) and the Sternheimer & Malbin stain, prepared as described by the authors (8). Trypan was prepared as 1% aqueous solution, the dye concentration in the filtrate being 0.18%.

The plasma proteins used were human albumin (20%), human fibrinogen (1%) and gammaglobulin (12%) (Kabi, Sweden).

An electronic particle counter Celloscope 101 (AB Lars Ljungberg, Sweden) was used.

METHODS

Cell morphology was examined in unstained preparations using phase contrast microscopy.

Cell recovery at 1 g was examined by counting with the Celloscope after careful mixing of the cell suspension; decrease in cell concentrations is due to irreversible aggregation (9).

Cell recovery at g was measured with the Celloscope as percentage of cells recovered after compared to before centrifugation. A standardized procedure was used: 1 ml cell suspension was centrifugated and remixed with a Pasteur pipette with rubber bulb.

Supravital staining of leukocytes was measured by repeated counting of the same areas in Bürker counting chamber, which had been sealed with immersion oil to avoid drying.

RESULTS

Fig. 1 shows that erythrocytes and leukocytes in saline 0.9% became irregularly shaped and the leukocytes tended to spread on the glass. A small trace of plasma, added to saline 0.9% was enough to protect the shape of erythrocytes and leukocytes. Similar results were obtained with addition of fibrinogen, albumin or gammaglobulin.

Table I shows that the recovery of erythrocytes after centrifugation was significantly higher than that of platelets or leukocytes. The recovery was also independent of the procedure used for washing of the centrifuge tube or the degree of centrifugation within the limits used (Table II). There was nearly total recovery of cells in plasma, while the recovery was sig-

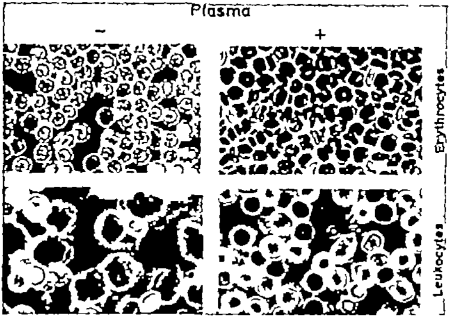


Fig 1. Erythrocytes and leukocytes in saline 0.9% with and without 1% plasma. Pictures taken after 10 min (× 592).

nificantly depressed in media not containing plasma.

Fig. 2 shows that platelets and leukocytes did not aggregate until after several hours in media with a trace of plasma. The decrease in cell concentration in media not containing plasma was found to be due to aggregation, this could also be observed macroscopically. Lysis of cells was ruled out by examining similar cell suspensions placed in Bürker counting chambers for six hours.

Figs. 3 *a* and *b* show that addition of plasma improved the recovery after centrifugation of platelets and leukocytes. Fig. 3 *c* shows that fibrinogen could maintain the cell recovery at smaller concentrations of protein than did albumin or gammaglobulin. A remarkably small trace of plasma (0.02%) was sufficient to improve the recovery of erythrocytes after centrifugation. The disparity of the results of cell recovery increased in media deprived of plasma proteins.

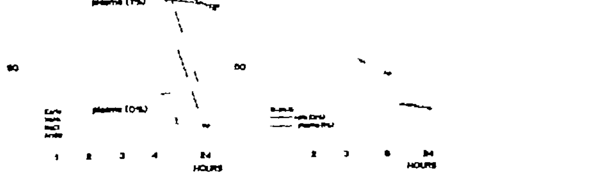


Fig. 2. Recovery of leukocytes and platelets in media with and without 1% plasma. The width of the shaded lines equals twice the intrasample variation. Each point is the mean of four samples. Cell concentrations 1.10^9 and $3.10^9/\mu l$ of leukocytes and platelets respectively

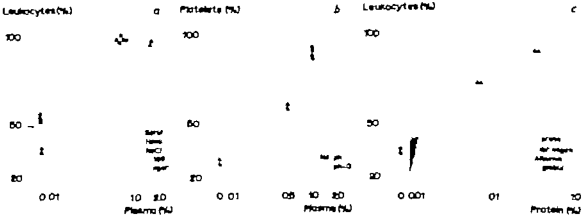


Fig. 3. Recovery of cells exposed to centrifugation (150 g for 10 min). Each point is the mean of four samples. The width of the shaded line equals twice the intrasample variation, cell concentrations 2.10⁸/μl.

Fig. 4 *a* shows that the dispersion of the results of supravital staining increased with the dilution of dye used, possibly because it was difficult to detect initial staining. Addition of plasma to the medium prevented leukocytes from being stained both with trypan (Fig. 4 *b*) and with the Sternheimer & Malbin stain (Fig. 4) similar protection against staining was obtained with plasma proteins.

DISCUSSION

In the absence of plasma proteins there was a tendency for blood corpuscles to become irregularly shaped, to aggregate, to show poor recovery after centrifugation and to become stained with supravital dyes. The addition of a small trace of plasma or plasma proteins was sufficient to protect the cells. In the absence of protein it has been shown that leukocytes might be attached to the glass walls of the centrifuge tube (11). Plasma proteins also protected leukocytes against the Sternheimer & Malbin stain, which has been shown elsewhere to be toxic to cells (11). The protection of cells against staining might be due to the possible protein binding of dyes, though this would not explain the protection of cell shape and the prevention of cells from being aggregated. It seems reasonable that the plasma proteins protect the cell membrane (5). Release of the plasma protein film attached to the cell membrane (5) might activate blood clotting enzymes, for instance the membrane-associated ATPase (3 4 7), leading to irreversible aggregation (9).

This protection of cells by protein may be of great clinical importance. Protein has been shown

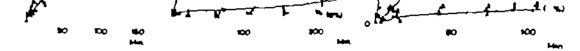


Fig. 4. Supravital staining of leukocytes in saline 0.9% (*a*), with and ithout plasma (*b* and *c*). The dye concentrations shown at (*a*) on figure; in (*b*) trypan 1/200, and in (*c*) Sternheimer & Malbin stain (1/10) were used.

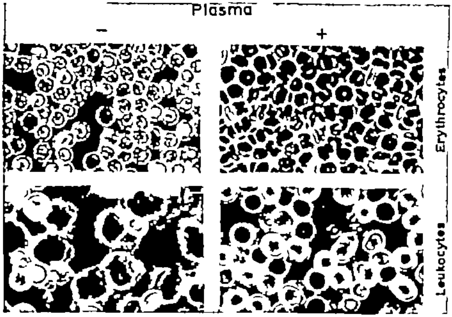


Fig 1 Erythrocytes and leukocytes in saline 0.9% with and without 1% plasma. Pictures taken after 10 min (592).

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Figs. 3 *a* and *b* show that addition of plasma improved the recovery after centrifugation of platelets and leukocytes. Fig. 3 *c* shows that fibrinogen could maintain the cell recovery at smaller concentrations of protein than did albumin or gammaglobulin. A remarkably small trace of plasma (0.02%) was sufficient to improve the recovery of erythrocytes after centrifugation. The dispersity of the results of cell recovery increased in media deprived of plasma proteins.

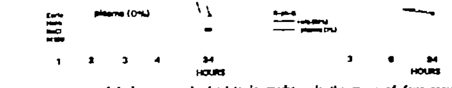


Fig. 2. Recovery of leukocytes and platelets in media with and without 1% plasma. The width of the shaded lines equals twice the intrasample variation. Each point is the mean of four samples. Cell concentrations 1.10^6 and $3.10^6/\mu l$ of leukocytes and platelets respectively

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INFLUENCE OF PROTEINURIA ON THE QUALITATIVE AND QUANTITATIVE EXAMINATION OF LEUKOCYTES IN URINE

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Abstract. Standard separated leukocytes from peripheral blood have been used to study whether protein in urine might interfere with the behavior of leukocytes. It was found that protein protected leukocytes from being aggregated and stained supravitally. A higher percentage of leukocytes became stained with the Sternheimer & Malbin stain than with other supravital dyes, possibly due to toxic influence from alcohol. It is supposed that the questionable difference between "pale" and "red" stained leukocytes with the Sternheimer & Malbin stain is related to the presence or absence of protein. Protein was also found to interfere with the quantitative examination of leukocytes, particularly in centrifugated urine specimens, since leukocytes became aggregated or attached to the glass walls in the absence of protein.

The qualitative study of leukocytes (*L*) in urine aroused a renewed interest in 1949 when Sternheimer and Malbin (17) found predominantly "pale stained" *L* in pyelonephritis and "red stained" *L* in cystitis. The "pale stained" *L* are probably not very different from the "glitter cells" found by Schilling in cases of pyelonephritis as early as 1908 (13, 18). The value of the Sternheimer & Malbin staining method has not been definitely established, some being in favor (2, 3, 4, 5, 9, 10, 11, 12, 25), others not (14, 15); some consider the "pale stained" *L* to be dead or dying (2, 12), most regard the "red stained" *L* as being dead.

The quantitative examination of *L* in urine, besides being related to the rate of urine flow, depends on the degree of aggregation and the exactness of the counting method. At present the direct counting methods are preferred in scientific works (1, 6), while examination of centrifugated urine specimens is used in practical clinical work.

In a previous paper (23) it was shown that plasma proteins maintained the shape of blood cells, prevented blood cells from aggregation and *L* from being stained supravitally. The purpose of the present paper was to study whether protein in urine might interfere with the behavior of *L*. Since *L* separated from urine specimens might be exposed to different factors interfering with their vitality, it was found preferable to use *L* of constant vitality separated from peripheral blood.

MATERIAL

Separation of polymorphonuclear leukocytes (PML) and lymphocytes (LC) from peripheral blood was done as described elsewhere (21); the contamination of erythrocytes and platelets was relatively small (E : P : L = 0.2 : 1 : 1). Some experiments were done with *L* separated from urine specimens using the centrifuge tube described elsewhere (21).

Urine specimens used in the qualitative study are shown in Table I. An attempt was made to render the differences between urine specimens as small as possible, except as regards protein; the pH was adjusted to 7.4 to avoid the influence of different pH (7, 24). EDTA (pH 7.4, 2 mg/ml) and glucose (1 mg/ml) were added ("standardized" urine specimens). In the quantitative study the urine specimens were not specially treated. All urine specimens were filtered through filter paper and through millipore filter (Schott G 4, Mainz, Germany).

Dyes. The dyes (Merck, Germany), were prepared as 1% aqueous solutions. The dye concentrations in the filtrate were: trypan 0.18, eosin Y 1.0, neutral red 0.09, safranine 0.09, gentian violet 0.009 and methylene blue 0.03%. The Sternheimer & Malbin stain was prepared as described by the authors (17).

An electronic particle counter Celloscope (Lars Ljungberg, Sweden) was used.

Table I. *Urine specimens*

C = Coli, *P* = Proteus, E = Enterococcus

Urine no.	Protein (mg %)	M. osm.	Bacteriuria	Remarks
1	101	360		Intox. gravid.
2	166	230	+C	Ca. vesica uria.
3	110	190		Hydroneph. & Urolith.
4	97	300		Febrile protein
5	0	360	+P	Cystitis
6	137	250	+E	Ca. vesic. urin.
7	0	150	+P	Cystitis
8	280	270	+P +E	Retentio urin.
9	156	190		Urolith.
10	1624	170		Nephrosis
11	0	220	+C	Ad. prostatae[a]
12	414	120	+P	Nephrit. chron.
13	143	260	+C	Ad. prostatae
14	0	300	+C	Cystitis
15	101	170	+	Ad. prostatae
16	6	280		Cystopyel. chron.
17	0	220		Hypertonia
18	0	85		
19	0	180		
20	0	425		
21	155	450	+P	Cystitis[a]
22	156	420	+P +E	Cystopyel. chron.
23	129	140	+P +C +E	Ad. prostatae[a]

[a] Permanent catheter.

METHODS

Vitality of L estimated from supravital staining. The cell and dye suspensions were mixed in the proportion 5 1. The following chamber technique was used: a small drop of the cell mixture was placed on a glass slide, two parallel bands of immersion oil were placed on each side of the field, a coverglass was placed on the slide and rimmed with immersion oil. The vitality was determined using the formula:

$$L_V = L_0 - (L_A + L_S) = L_F - (L_S/L_F)\,L_0$$

Since it was difficult to measure L_A and L_S directly this was obtained indirectly $L_A = L_0 - L_F$ and $L_S = (L_S/L_F)\,L_0$. Total (L_0) and free L (L_F) were determined by counting with the Celloscope. L_S was measured by counting stained L_F within 5 min; means of 400 cells were used. L_0 was set to equal 100% vitality

Vitality of L estimated from O_2-consumption. The Cartesian diver technique was used; both standard and ampulla standard divers were used (22). The O_2-consumption of 10^{-10} L from time 0 to t

$$Y = \frac{V_{air} \times \Delta p \times 273 \times 10^{10}}{10\,000 \times 310 \times L_d} = k\Delta p$$

where V_{air} is the air volume of the diver Δp the mm change of manometric pressure from time 0 to t, 10 000 the mm Brodie fluid which equal 1 atmosphere, L_d the leukocytes/diver. The corresponding Y and t values were transferred to a computer. Since the O_2-consumption of L in urine usually decreases, the regression equation of second degree was preferable to that of first degree; the derivated regression equation was used to measure vitality at any time (Y'), while $\int^{tn} Y\,dt$ was used in comparative calculations. The O_2-consumption of L in plasma (1 900 μl 10^{-10} L h^{-1}) was set to equal 100% vitality (22).

Recovery of L in urine specimens not exposed to centrifugation was measured with the Celloscope a decrease in cell concentration is due to irreversible aggregation (20).

Recovery of L in urine specimens exposed to centrifugation (150 g for 5 min) was determined by transferring the mixed centrifugate to a counting beaker for counting with the Celloscope the mixed centrifugate is the residue of fluid in the bottom of centrifuge tubes after decantation.

Volume of mixed centrifugate was determined by using suspension of erythrocytes with known cells/μl; this method does not include the compact cell column after centrifugation, which may differ according to the cell concentrations in urine specimens.

Statistical calculations. The comparison between paired groups was done with the Wilcoxon two-sample test; the correlation coefficients were calculated according to the Spearman rank correlation test, and comparisons between several means with equal sample size by the Student's range test.

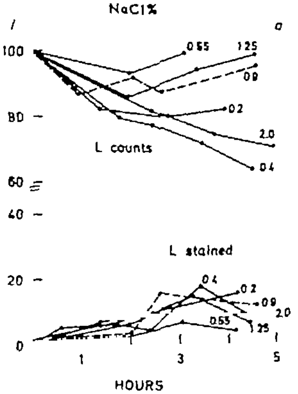


Fig. 1. PML were suspended in saline solutions of different osmolarities; the upper and lower curves show respectively the percentages of PML aggregated and stained supravitally by trypan.

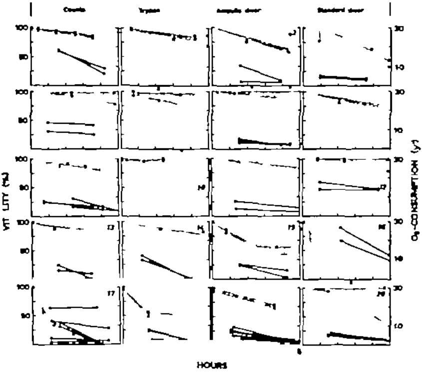


Fig. 2. PML separated from blood were suspended in different urine specimens. The upper curve show free L (L_F), the shaded area the supravitally stained L (L_S) using trypan; vital L (L_V) correspond to the lower curve of this area. The O_2-consumption is shown by Y" with standard (●—●) and ampulla standard divers (O—O). The numbers correspond to those in Table I.

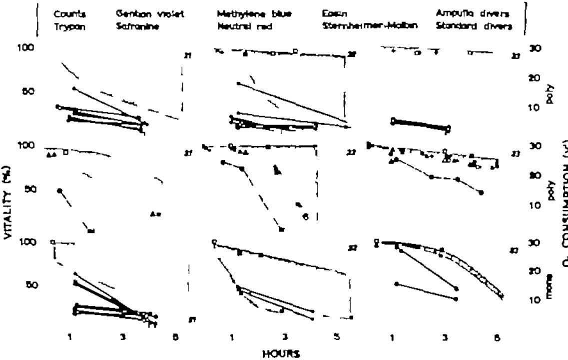


Fig. 3. The curves correspond to those in Fig. 2 comparing PML and LC. The different dyes used in supravital staining are marked.

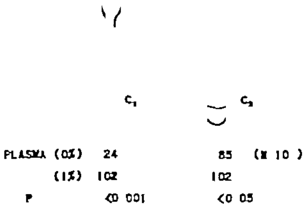


Fig. 4. Recovery of PML in saline 0.9% with and without 1% plasma in conical and cylindrical centrifuge tubes. Means of six samples.

RESULTS

Qualitative examinations of L in urine. Fig. 1 shows that the aggregation and staining of *L* were slightly increased in solutions differing from normal osmolarity· this may slightly interfere with our results, since the urine specimens differed in osmolarity

The percentages of *L* which were aggregated and stained (L_A and L_B) differed markedly in different urine specimens (Figs. 2 and 3). A significant correlation was found between vital *L* (L_T) after 4 hours and quantity of protein in urine specimens (−0.46, $p < 0.05$). Fig. 3 shows good agreement between the different dyes used in supravital staining. A higher percentage of *L* were stained with the Sternheimer & Malbin stain than with other dyes gentian violet and safranine in aqueous solutions, which are the active dyes of the Sternheimer & Malbin stain, showed results similar to other dyes used in supravital staining. The percentage of LC which were stained was slightly higher than of PML (Fig. 3) in the same urine specimens.

The O_2-consumption of *L* separated from urine specimens showed a great dispersion of results (Table II)· the O_2-consumption might be several times higher than vital *L* separated from blood. These strange results are due to *L* being aggregated during separation from urine, which made determinations of cell concentrations impossible. The vitality of *L* judged by O_2-consumption was nearly always depressed compared to vitality judged by supravital staining (Figs. 2 and 3).

Quantitative examinations of L in urine The

Table II. *O_2-consumption of PML separated from blood (A and B) and from urine (C)*

Source of PML	L medium	O_2-consumption[a] Mean	Range	N
A	Plasma	147	140–153	5
B	Urine	35	17 52	4
C	Urine	131	20–550	13

[a] γ dr cm³ h⁻¹ 10⁻¹⁰ PML

Table III. *Recovery of leukocytes from different urine specimens*

Urine Protein (mg %)	M. osm.	pH	Recovery of leukocytes (%) min[a]	1 g	g[b]
0	430	6.6	85	84	23
0	200	6.6	120	85	3
0	150	5.2	150	93	70
50	600	5.2	97	90	80
100	500	5..	85	97	94
100	360	6.6	180	78	71
110	200	6.6	125	87	83
143	260	5.2	163	92	80
156	420	5.2	130	94	57
280	270	6.4	145	95	70
Allowance (p − 0 05)				10	14

[a] The time leukocytes were suspended in urine at 1 g
[b] With ct-tubes (100 g, 5 min). Each number is the mean of replicate counts of three parallel samples.

Table IV *The volume of mixed centrifugate*

Exp.	Mixed centrifugate (µl)	S.E.	N
1	196	3.3	10
	88	4 0	10
3	60	..6	10

10 ml cylindrical tubes were used. Exp. 1 The tubes were decanted. Exp. 2: As 1 + fluid was dried off from the rim. Exp 3 As 1 + tubes were inverted on filter paper for 10 sec.

mean recovery of *L* suspended in 10 different urine specimens for 2–3 h (Table III) was usually not significantly depressed if they had not been exposed to centrifugation, but was depressed after centrifugation, particularly in the absence of protein in urine. Fig. 4 shows that the recovery was markedly improved with cylindrical compared to conical centrifuge tubes in the absence of protein, while the difference was small in the presence of protein.

The volume of the mixed centrifugate was found to depend on the decanting time and on whether superfluous fluid was dried off (Table IV).

DISCUSSION

There was no difference in supravital staining with different dyes, although the dye concentrations differed greatly. However the dye concentrations might interfere with the results if too high dilutions are used (23) or if the dye is toxic to cells (18). The methods used for measuring vitality of cells possibly differ in sensitivity and exactness. A similar difference as was found between vitality judged by O_2-consumption, and supravital staining has been found between supravital staining and cell cultivation (8). One author considers the stained cells to be dead and unstained cells not necessarily to be alive (16). So far the vitality judged by supravital staining has been judged from the percentage of free *L* which are stained, without taking into account aggregated *L*, this may sometimes lead to erroneous results, since stained *L* were found to be more liable to aggregate, whereby a high percentage of stained *L* may be changed into a small percentage of stained *L* although the vitality is decreasing. The method used in this work may theoretically be somewhat incorrect, since aggregated *L* may not all be dead, furthermore aggregates are included in the measurement of free *L*, though these errors are small and the method is the most exact possible.

Concerning the qualitative examination of *L* in urine, it was found that the Sternheimer & Malbin stain showed an additional staining compared to other supravital dyes, due to the possible toxic influence of alcohol. However protein was found to greatly interfere with the supravital staining, and it therefore seems reasonable to attribute the different staining property of *L* with the Sternheimer & Malbin stain to the presence of protein. Since the quantity of protein in urine may depend on the degree of infection, this may explain the different staining of *L* in pyelonephritis and cystitis. However the additional toxicity of the Sternheimer & Malbin stain decreases the value of this staining method if aqueous solutions are not used. This influence of protein also explains why "pale stained" *L* are found in other conditions where protein is present, for instance in cases of meningitis (18) or in the presence of plasma proteins (23).

In the quantitative examinations of *L* in urine, the direct counting methods were found to have the great advantage that aggregation was small both in the presence and in the absence of protein. Examinations of centrifugated urine specimens have the advantage of concentrating the cells, which decreases the counting error at small cell concentrations. The error due to aggregation, adhesion of *L* or different volume of mixed centrifugate, may be decreased with a proper technique, for which reason examination of mixed centrifugate may be the preferred method in the differentiation between normal and pathological urine.

Protein in urine probably acts by protecting the vitality of *L*, this relationship in urine does not seem previously to have been described. The protecting influence of protein may even be essential in maintaining the phagocytotic activity of *L*.

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TRACE ELEMENT BALANCES IN TWO CASES OF PANCREATIC INSUFFICIENCY

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Abstract. Balance studies in two patients with pancreatic insufficiency have been performed with respect to the following trace elements: Ag, As, Au, Br, Cd, Cr, Co, Cu, Fe, Hg, La, Mo, Rb, Sb, Sc, Se, Sm, W and Zn. The method consisted in neutron activation analysis combined with a recently developed ion exchange technique. The results suggest that the metabolism of trace elements with both known, suspected and unknown biological function is influenced by exocrine pancreatic enzymes.

Trace element metabolism in relation to exocrine pancreatic function has previously received some attention. The zinc content of pancreatic secretions has been found to be lower in cases of chronic pancreatitis than in normals—a difference that distinguished these two groups more clearly than did the bicarbonate production (10).

Iron absorption in pancreatic insufficiency of various origins has been thoroughly investigated before and after substitution therapy with pancreatic enzymes. Some of these studies suggest that iron absorption is depressed by the presence of pancreatic secretions but enhanced by their absence (1, 4, 5). This increase in absorption has, however, been tentatively ascribed to associated liver damage (7).

The behaviour of other trace elements in relation to pancreatic exocrine function has not received the same attention, and the possibility that they are influenced by pancreatic secretions has not hitherto been explored.

By means of a recently developed ion-exchange technique, applied after neutron activation, a large number of trace elements can be assayed simultaneously (9, 15).

The aim of the present study was to examine the trace element metabolism by means of full balance investigations in two cases of pancreatic insufficiency before and after substitution therapy with pancreatic enzymes.

MATERIAL AND METHODS

Two patients, A and B, with pancreatic insufficiency were examined with regard to the over-all balance of trace elements. Patient A had chronic pancreatitis and patient B had undergone total pancreatectomy.

Patient A. A 52-year-old official with a long history of attacks of vomiting, diarrhoea and epigastric pain. Repeatedly admitted to hospital with the diagnoses of acute gastritis, gastroenteritis and acute pancreatitis. Frequent abuse of alcohol. Fracture of the ribs on at least 20 occasions. At the age of 40 a pancreatic cyst removed. Prior to present admission increasing loss of appetite and weight, with long period of diarrhoea. Pancreatic insufficiency diagnosed. Severe steatorrhoea, with a maximum faecal fat level of 85 g/day. D-xylose test normal. Mild macrocytic anaemia with Hb level ranging from 11.9 to 10.5 g%. MCV 120 μ^3. Vitamin B_{12}, folinic acid, calcium, phosphorus and cholesterol in serum normal, serum magnesium and prothrombin-proconvertin activity slightly low.

The trace element analyses in patient A were performed under the following conditions: I. Normal hospital diet. II. As I, but supplemented with 22 tablets of Pankreoyar® per day (200 mg of pancreatin per tablet). The trace element concentration in serum was determined in the middle of each period.

Patient B. A 62-year-old gardener, cholecystectomized some years previously for uncomplicated gall-stones. Apart from mental depression, previously in generally good health. No diabetes or history of alcohol abuse.

Five years previously abdominal pains; examination suggested pancreatic carcinoma. Total pancreaticoduodenectomy and resection of the lower two-thirds of the stomach. No histological confirmation of carcinoma.

Since the operation 40 U of insulin per day; diabetes not particularly troublesome. For the exocrine pancreatic insufficiency various enzyme preparations were given.

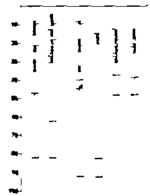


Fig. 1. The fat balances of patients A and B for various periods.

The weight, which had dropped by 15 kg during the first two years after operation, then remained constant, and the nutritional state was satisfactory with no clinical evidence of malabsorption apart from the steatorrhoea.

The trace element balance studies in patient B were performed under the following conditions: I. Normal hospital diet containing 82 g of fat per day. II. As I, but supplemented with 800 mg of Epase per day (64 000 U.). III. Normal hospital diet containing 48 g of fat per day supplemented by 9 tablets of Pankreozym® per day (200 mg of pancreatin per tablet). IV. As III, but, instead of Pancreozym® supplemented by 9 tablets of Nutrizym® per day (400 mg of pancreatin and 50 mg of bromelin per tablet). The trace element concentration in serum was determined in the middle of periods I and II.

Balance technique. The patient was kept on a constant diet prepared in the metabolic ward and the faeces and urine were collected for balance periods of five days. The conventional carmine-labelling technique was applied (6). The amounts of the trace elements in the urine and faeces for each balance period were determined on aliquots of urine and homogenates of faeces by the method described below. The intake of the trace elements was determined by analysis of homogenates of duplicates of the daily menu. All the drugs were added to the model diet before homogenization. The balance values obtained were charted as described by Reifenstein et al. (8).

Neutron activation analysis. The collected specimens were stored in polythene bags at 20°C. When required for analysis, aliquots were transferred to quartz ampoules, which were weighed dried and sealed as previously described (8). The ampoules were then irradiated together with standards for 24 h in the R-2 reactor at Studsvik with thermal neutron flux of $2 \cdot 10^{12}$ n/cm²/s. A decay interval of 2 days lapsed before chemical processing. The chemical separation of the elements included decomposition of the organic material with sulphuric acid and hydrogen peroxide, distillation step with hydrobromic acid, and separation on various ion exchange columns into 14 groups of elements (9–13, 15).

The γ-spectrometric measurements were carried out with a transistorized 512-channel pulse-height analyser attached to 3×3 NaI (Tl) well-type crystal. The identification and the quantitation of the elements have also been described elsewhere (12, 13).

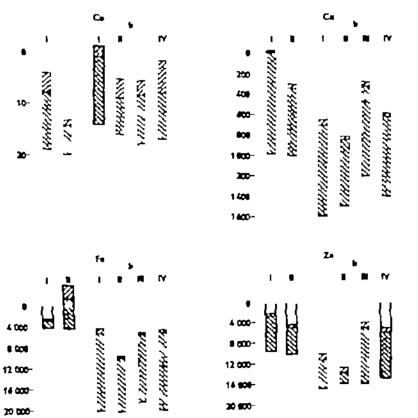


Fig. 2. The balances of the trace elements with known biological function.

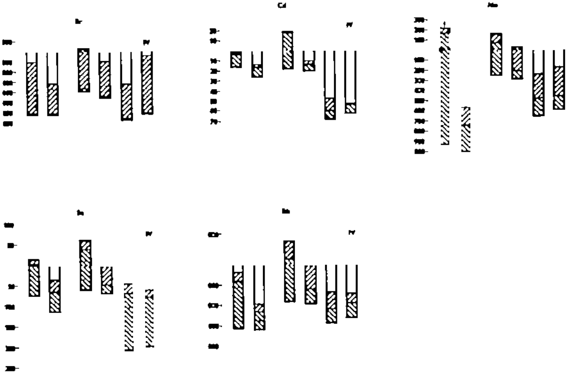


Fig. 3 The balances of the trace elements with suspected biological function

RESULTS

The intake and excretion of Ag, As, Au, Br Cd, Ce, Co, Cs, Cu, Fe, Hg, La, Mo Rb, Sb, Sc, Se, Sm, W and Zn by the two patients with pancreatic insufficiency are listed in Tables I–III and charted in Figs. 2–4 The trace element concentration in serum of the two patients during periods I and II is presented in Table IV The fat balances for the various periods of patients A and B are shown in Fig. 1 When no exocrine pancreatic substitution was performed, there was substantially more fat in the faeces.

The trace elements with a known biological function are given in Table I and Fig. 2, those with suspected biologic function in Table II and Fig. 3 and those with no known biological function in Table III and Fig. 4

So far as the trace elements with *known biological function* are concerned, patient A had a slightly negative Cu balance without substitution and a positive Cu balance with the Co balance was slightly more positive during substitution. The Fe balance, on the other hand, was positive when no substitution was performed and otherwise negative. The Zn balance was positive both without and during substitution. Patient B had positive Cu, Fe and Zn balances both without and with the various enzyme preparations added. The Co balance was slightly negative without substitution and positive with the various forms of substitution therapy

Among the trace elements with *suspected biological function* in patient A, Mo and Se showed negative balances without substitution and positive with, and Br, Cd and Rb recorded positive balances that became more positive during substitution. Patient B had negative balances of all the elements in this group without substitution. In the case of substitution therapy with Nutrizym® and Pankreozym® all the elements showed positive balances. With lipase the Br and Cd balances were positive and those of Mo, Rb and Se almost zero.

Owing to a technical mishap the balances of the trace elements with *no known biological function* were not complete for all the periods. Among

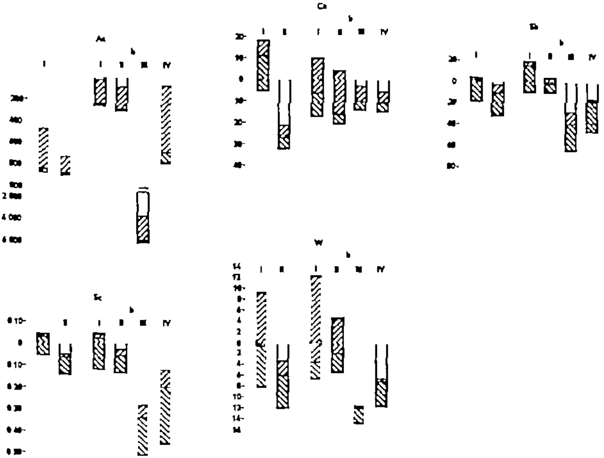


Fig. 4 The balances of the trace elements with no known biological function.

those that were complete—As, Cs, Sb, Se and W—all but As showed in both patients negative balances without pancreatic substitution and positive balances with. In the case of lipase the balances of Cs and W in patient B remained negative.

The concentrations of the trace elements in serum (Table IV) did not differ appreciably for the various periods. The concentration of Fe in patient A was, however lower with substitution than without.

DISCUSSION

The present balance study was performed in the conventional way with analyses of the intake and excretion in the faeces and urine, possible losses via the skin and sweat being disregarded. Another conceivable source of error lies in the carmine labelling technique, but this is negligible since an analysis of a carmine capsule showed it to contain extremely little of the trace elements in relation to the amount ingested or excreted.

Balance studies of trace elements in healthy subjects (8, 12, 13) and in cases of osteomalacia (12, 13) have been reported, but none appears to have been made in cases of pancreatic insufficiency. When no pancreatic substitution was performed, both patients in this study excreted large amounts of faecal fat, while during this therapy the steatorrhoea decreased greatly (Fig. 1). The faecal excretion of fat in patient B was about the same for the different enzymic preparations. The nitrogen balance, however which was examined only during periods III and IV tended to be more positive in the former (Nutrizym®) than in the later (Pankreozym®).

Several of the trace elements showed negative

balances in the periods without exocrine pancreatic substitution, but otherwise positive. This applies to antimony cesium, copper molybdenum, scandium, selenium and wolfram in patient A and to bromine, cadmium, cobalt, rubidium and scandium in patient B. The balances of bromine, cadmium, cobalt and rubidium in patient A showed a similar tendency being more positive when substitution therapy was given than when it was not.

The iron balance in patient A was the only one that was positive without exocrine pancreatic substitution and negative with. This is consistent with the reports that pancreatic secretions depress iron absorption. In long-time substitution therapy with pancreatin patient A also developed sideropenic anaemia, the fact that patient B did not show a negative iron balance on substitution may be due either to the smaller amounts of pancreatin administered to this patient or to the absence of any evident liver damage (7)

The balances of most of the trace elements were positive for substitution with the various enzymatic preparations given to patient B. Only in the case of lipase substitution were some balances negative (antimony cesium, molybdenum and wolfram). No marked differences between the periods on Nutrizym® and Pankreozym® were discerned. With the exception of iron in patient A the serum values recorded in Table IV did not differ appreciably between periods with and without substitution. Most of the values are consistent with the normal values of trace elements in plasma compiled by Bowen (3).

In conclusion, this investigation has shown that the metabolism of trace elements with both known, suspected and unknown biological function is influenced by pancreatic exocrine function.

Table I. *Trace elements with known biological function (μg/24 h)*

Element	Patient	Period	Daily intake	Excretion		Balance[a]
				Faeces	Urine	
Co	A	I	19	11	3.8	+4.2
		II	20	5.5	1.0	+13.5
	B	I	14	13	2.2	−1.2
		II	16	7.0	3.7	+5.3
		III	18	9.5	2.8	+5.7
		IV	17	8.9	6.3	+1.8
Cu	A	I	980	980	32	−32
		II	1 000	660	42	+304
	B	I	1 600	890	61	+649
		II	1 500	660	23	+817
		III	1 200	880	40	+280
		IV	1 400	780	34	+586
Fe	A	I	4 200	1 300	160	+2 740
		II	4 400	5 600	2 700	−3 900
	B	I	20 400	14 900	750	+4 750
		II	20 500	10 100	310	+10 090
		III	18 800	13 000	70	+5 730
		IV	20 500	15 000	280	+5 220
Zn	A	I	9 400	6 700	500	+2 200
		II	10 000	5 200	430	+4 370
	B	I	17 000	5 500	1 200	+10 300
		II	16 000	2 000	1 100	+12 800
		III	16 000	11 000	1 000	+4 000
		IV	15 000	9 000	900	+5 100

[a] Intake minus excretion.

Table II. *Trace elements with suspected biological function (μg/24 h)*

Element	Patient	Period	Daily intake	Excretion		Balance
				Faeces	Urine	
Br	A	I	6 200	39	5 000	+1 161
		II	6 300	55	3 100	+3 145
	B	I	3 900	220	3 900	−220
		II	4 600	30	3 600	+970
		III	6 800	15	3 500	+3 285
		IV	6 200	11	5 700	+489
Cd	A	I	16	12	2.1	+1.9
		II	26	10	2.4	+13.6
	B	I	18	36	1.0	−19
		II	20	6.1	3.1	+10.8
		III	68	8.8	12	+47.2
		IV	62	8.1	1.0	+52.9
Mo	A	I	930	1 100	44	−214
		II	1 000	260	170	+570
	B	I	250	330	80	−169
		II	280	77	230	−27
		III	650	170	240	+240
		IV	590	130	290	+170
Rb	A	I	6 300	4 600	850	+850
		II	6 400	1 900	790	+3 710
	B	I	3 700	4 200	1 700	−2 200
		II	3 900	1 400	2 400	+100
		III	5 800	1 300	1 700	+ 800
		IV	5 300	1 400	930	+2 970
Se	A	I	71	73	11	−13
		II	110	46	30	+34
	B	I	65	101	23	−59
		II	70	22	48	± 0
		III	210	140	23	+47
		IV	200	120	15	+65

Table III *Trace elements without known biological function (μg/24 h)*

Element	Patient	Period	Daily intake	Excretion		Balance
				Faeces	Urine	
Ag	A	I	16	97	0.82	−81.82
		II	16	—	0.86	—
	B	I	3.6	2.4	1.0	+0.2
		II	1 4	0.88	—	—
		III	—	—	—	—
		IV	—	—	—	—
As	A	I	870	26	370	+474
		II	900	12	150	+738
	B	I	260	10	230	+20
		II	310	1 7	220	+88.3
		III	6 200	96	2 300	+3 904
		IV	790	89	620	+81
Au	A	I	0.0087	—	0.0032	—
		II	—	0.72	0.032	—
	B	I	0.048	0.034	0.0077	+0.01
		II	0.087	0.018	0.0045	+0.06
		III	0.53	0.00072	0.088	+0.44
		IV	0.36	—	0.029	—
Co	A	I	16	—	—	—
		II	—	26	—	—
	B	I	16	7.5	1.3	+7
		II	17	0.85	5.3	+10.85
		III	180	39	22	+119
		IV	240	28	17	+195
Cs	A	I	5 0	16	7.0	−18
		II	32	4 7	6 0	+21
	B	I	17	11	16	−10
		II	20	3.4	21	−4.4
		III	14	3.6	7.2	+3.2
		IV	15	4 2	5.1	+5 7
Hg	A	I	—	—	—	—
		II	—	—	—	—
	B	I	4 6	4.1	3.5	−3 0
		II	4.0	2.9	2.4	−1.3
		III	24	11	3.3	+9 7
		IV	30	15	0.6	+14 4
La	A	I	8.4	9 7	—	—
		II	7.2	8.8	0.15	−1 8
	B	I	5.9	6.2	1 0	−1.3
		II	4.5	2.6	1.8	+0.1
		III	6.0	3.3	0.69	+2.0
		IV	4.5	1.6	0.97	+1.9
Sb	A	I	18	19	1 4	−2 4
		II	32	21	7.8	+3.2
	B	I	9 1	20	3.5	−14 4
		II	11	7 7	4.9	−1 6
		III	65	25	11	29
		IV	47	28	1.0	+18
Sc	A	I	0.050	0 083	0 0081	−0 04
		II	0.14	0.075	0.01	+0.05
	B	I	0.12	0.14	0.019	−0 04
		II	0.14	0.076	0.029	+0.03
		III	0.52	0.17	0.057	+0 29
		IV	0 47	0.26	0 079	+0.13
Sn	A	I	18	48	0 1	−30.1
		II	20	15	0.53	+4.47

Table III (*Continued*)

Element	Patient	Period	Daily intake	Excretion		Balance
				Faeces	Urine	
	B	I	54	29	2.3	+22.7
		II	68	10	0.2	+57.8
		III	37	13	3.3	+10.7
		IV	28	15	1.2	+11.8
W	A	I	8.2	7.8	11	−10.6
		II	12	6 1	2.5	+3.4
	B	I	6.8	3.3	19	−15.5
		II	5.6	3.6	6.4	−4.4
		III	15	2.9	0.22	+11.88
		IV	12	4.7	0.52	+6.78

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Table IV *Trace element concentration in serum (μg/ml)*

		Patient A		Patient B	
		Period I	Period II	Period I	Period II
Trace elements					
Known biological function	Co	—	0.0034	0.0011	0.0013
	Cu	1 4	1.3	0.77	0.77
	Fe	1 4	0 61	2.1	1.9
	Zn	0.95	0.80	1.2	1 1
Suspected biological function	Br	7.3	7.3	1.8	1.8
	Cd	0.005	0.002	0.001	0.005
	M	—	0.010	0 012	0 007
	Rb	0.26	0.25	0.22	0.19
	Se	0.05	0.08	0.10	0.09
Not known biological function	As	—	0.010	0.003	0.003
	Au	0 000008	0 00002	0.000002	0.000004
	Cs	—	—	0.01	0.01
	Cs	0.0018	0.0015	0 0021	0 0024
	Hg	0.003	0.0002	0.0007	0.0009
	Sb	—	0.003	0.0007	0 0007
	Sc	0.00002	0.000006	0.000006	0 000007
	Sn	0.002	—	0.0003	0.0002
	W	—	0 008	0.007	0.005

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Quinidine sulphate in sustained release form
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"Comparative study of a long acting quinidine preparation and quinidine sulphate in chronic atrial fibrillation'

Török, E., Bajkay G., Gulyás A. & Makláry E. Pharmacol. Clin. 2:90 1970.

During a period of 15 months a comparative study between Kinidin Durules® and ordinary quinidine sulphate tablets was performed. 26 patients with atrial fibrillation were DC-reverted and subsequently treated with quinidine as maintenance therapy The aim of the study was to obtain answers to the following questions

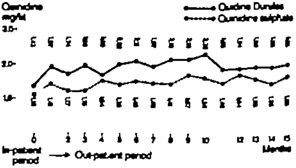
- Is there any difference in the therapeutic value of Kinidin Durules® and ordinary quinidine sulphate tablets in the maintenance of sinus rhythm after DC-shock in patients with atrial fibrillation?
- Which of the two preparations gives the least side effects?
- Is there any difference between the two presentations in the maintenance of stable and sustained plasma quinidine levels?

The study gave the following results

- In the maintenance of sinus rhythm the two preparations were found to be equivalent.
- Quinidine sulphate tablets caused side effects more frequently than the long-acting *Kinidin Durules® did. Gastrointestinal* symptoms were observed in five patients in the quinidine sulphate group but only in one in the Durules® group
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The authors conclude

Our study supports the superiority of the long-*term treatment with* Kinidin Durules® as compared to quinidine sulphate for the maintenance of sinus rhythm. The antiarrhythmic potency of the Durules® is equal to that of the quinidine sulphate while the need of only two doses daily is more favourable for the patient and ensures a more stable plasma quinidine level. That in turn might explain the less frequent occurrence of side effects and especially of gastrointestinal complaints.



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Acta med. scand. Vol. 190 pp. 163–173 1971

THE EFFECT OF FUROSEMIDE ON THE FUNCTION OF NORMAL AND DISEASED KIDNEYS IN PATIENTS WITH UNILATERAL PYELONEPHRITIS

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Abstract. In nine patients with unilateral pyelonephritis the renal function has been measured by the split function technique before and after the i. injection of furosemide. Before the injection the excretion fraction of sodium and chloride as well as the diuresis, the osmotic clearance and the free water clearance per unit of glomerular filtrate were greatest, and the urinary concentration of inulin was least, in the pyelonephritic kidneys. After the injection of furosemide these differences were no longer significant. When related to the osmotic clearance, the free water clearance was reduced in all kidneys after the injection. The findings are compatible with the presence of functioning loop of Henle in most nephrons of the pyelonephritic kidneys.

The purpose of this study was to test the hypothesis of *intact nephrons* (10 12). In its original version (12) this hypothesis claims that in renal disorders with loss of nephrons, such as chronic glomerulonephritis, chronic pyelonephritis, and nephrosclerosis, the renal function is maintained by nephrons which are functionally intact. If this is true, a diuretic might be expected to have a similar effect upon normal and diseased kidneys.

In the present study the effect of furosemide upon patients with unilateral pyelonephritis has been investigated by the selective renal function technique. This diuretic was chosen because 1) it exerts its main effect upon a well-defined part of the nephron, the loop of Henle (5 9 15 25 32, 41 48), and 2) loss of some functions of this segment cannot be compensated for by other parts of the nephron. The obvious experimental advantages of the identical and normal milieu interieur for each pair of kidneys investigated have been discussed in detail by others (10).

A similar study in man has not been published before. In split-bladder dogs with one "pyelonephritic" kidney the fractional excretion of sodium increased in both kidneys after the injection of furosemide, but the characteristic difference between the kidneys persisted after the injection (44)

MATERIAL

Nine patients with *unilateral pyelonephritis* were investigated. A kidney was considered as not affected when it was without clinical symptoms and signs of renal disease and when the findings on macroscopy and bacteriological examination of the urine, and on intravenous pyelography and—in most patients (Table I)—renal arteriography were normal. The average length of the non-affected kidneys was 12.6 cm. The length of all these kidneys was *within* *standard deviations from the* normal mean value (34), i.e. gross compensatory hypertrophy was not present.

Roentgenological findings signifying loss of renal tissue in the affected kidney were criterion for acceptance in the study because nephron loss was an essential presumption of the hypothesis to be tested.

The average difference between the length of the normal and the diseased kidney in the same individual was 2.1 cm (1.0–4.2 cm). The relevant clinical findings are given in Table I. Conventional criteria (53) of the symptoms and signs of pyelonephritis have been used. The biopsies were all surgical, and urine from the individual kidney was cultivated for bacteria in all patients. The patients with an elevated arterial blood pressure were all of the benign hypertensive type (39) with eyeground findings corresponding to groups I or II of Keith and Wagener and with no or slight left ventricular hypertrophy In the patients with pyelolithiasis, hydronephrosis and delayed emptying of the renal pelvis were absent.

Additional patients with *renal functional patterns* different from that of pyelonephritis were investigated. man, 57 years old, with *unilateral hydronephrosis*, and woman, 64 years old, with reduction of the renal parenchyma due to necrotic, poorly vascularized *renal cell carcinoma*, which probably did not influence the functional pattern of the kidney remnant (35).

Table I. *Relevant clinical data of the patients with pyelonephritis*

L = left kidney R = right kidney B = bladder + = presence, − = absence of findings signifying pyelonephritis. The ambulatory blood pressures are given

Pat. no.	Age (y)	Sex	History	Biopsy	Sediment	Bacteruria	Pyelogram	Angiogram	Scannogram	Pyelolithiasis	B.P (mmHg)
1	54	♂	L+	L+	B+	B−	L+ R−	L+ R−		L+	210/130
2	52	♀	+	R+	B+	B+	R+ L−	R+ L−	R+ L−	R+	110/70
3	65	♀	R+		B+	B−	R+ L−	R+ L	R+ L		210/130
4	31	♀	L+	L+	B+	B+	L+ R−				135/80
5	62	♀	L+	L+	L+ R−	L+ R−	L+ R−	L+ R−	L+ R−	L+	180/95
6	57	♀	−	L+	B−	B+	L+ R−		L+ R−	L+	250/115
7	61	♀	+		B+	R+ L−	R+ L−		R+ L−		160/90
8	66	♀	+		B+	B+	L+ R−	L+ R−	L+ R−		210/115
9	47	♀	+		B−	B−	R+ L−	R+ L−			210/120

Small infarction in upper pole.

All patients were without symptoms f circulatory insufficiency and the serum electrolytes were normal.

METHODS

The patients were kept on regular hospital diet and had not been given diuretics or antihypertensive drugs for the last week before the investigation. They fasted for approximately 12 h, and drank 1 000 ml water one hour before the investigation started. During the investigation water corresponding to the diuresis was given.

With the patient in the lithotomy position, which was maintained throughout the investigation, ureteral catheters (Carrière no. 6), with multiple side holes arranged in spiral for length of 10 cm, were introduced about 12 cm into the ureters. An indwelling Foley catheter was placed in the bladder. The male urethra was anesthetized with 2% lidocaine. No other anesthesia or premedication was given.

The catheterization was immediately followed by infusion of *p*-aminohippuric acid (20%) and inulin (5%). The sustaining doses were given in isotonic saline at rate of about 4 ml/min. The collection of urine for analysis began 30 to 40 min after the priming injection. After 2 to 3 control periods of about 10 min, 10 mg of furosemide (Lasix®) was injected intravenously in the course of 10 sec. The urine was then collected for 3 to 4 additional periods varying from 5 to 10 min, the "wash-out period" immediately following the injection of furosemide lasting about 10 min. During the investigation 5 to 7 samples of venous blood were taken.

Complete relaxation, freedom from pain or significant discomfort during the investigation, and diuresis of more than 2 ml/min given by regular peristaltic waves

Table II. *Protocol of a typical experiment* (*pat* 7)

The serum values (Na 137.5 mEq/l, Cl 110 mEq/l, osmolality 293.3 mosmol/kg H_2O U 29 mg%) used in the calculations were mean values of four samples taken during the experiment. The mean values of periods 2 3 and 4–5 are given in Table III. $V_K = U_K$. Diuresis. D = diseased, *N* = normal kidney

Time		Diuresis (ml/min) D	Diuresis (ml/min) N	C_{In} (ml/min) D	C_{In} (ml/min) N	C_{PAH} (ml/min) D	C_{PAH} (ml/min) N	U_{Na} (mEq/l) D	U_{Na} (mEq/l) N	U_{Cl} (mEq/l) D	U_{Cl} (mEq/l) N	U_K (mEq/l) D	U_K (mEq/l) N
13.10	Ureter catheters in position			13.15 Infusion of PAH and inulin started									
13.15–14 05		0 8	6.2										
14.05–14.15	1st clearance period	1.0	7 3	6.4	59	32	261	25	19	18	12	2 7	2 4
14.15–14.25	2nd clearance period	1.0	8.0	5 4	58	32	270	27	19	20	16	2 6	2.3
14.25	Injection of 10 mg furosemide i.v												
14.25–14.37	3rd clearance ("wash-out") period	1.5	9.2	8.0	50	41	213	27	49	24	44	2.7	3.1
14.37–14 42	4th clearance period	2.6	16.2	8.5	59	35	206	86	106	82	100	4.2	5.2
14 42–14.47	5th clearance period	2.2	15.0	8.0	58	23	160	97	96	92	98	4.6	4 6

from the best kidney during the control periods were criteria for inclusion in the study. Leakage from the ureter of the healthy kidney to the bladder occurred in patient 9: after repeated injections of Evans blue (1%) into the ureters, the dye appeared in the bladder only after the injections into the ureter of the healthy kidney. In this patient the values of urinary concentrations and clearances per unit of glomerular filtrate used in calculations of the relationship between the two kidneys were based upon analyses of the urine from the ureteral catheters.

Sodium and potassium in serum and urine were determined with an Eppendorf flame photometer; chloride was determined by the method of Lehmann (27), the electrical titration being carried out with an automatic titrator (Radiometer); the osmolality was determined with the Advanced Research Osmometer model 31-RAS, *p*-aminohippuric acid was determined by the method of Smith et al. (45), and inulin by the method of Walser et al. (50); urea was determined by the method of Conway (14).

The figures given in Table III are based upon the mean values of the two periods before the injection of furosemide (control values) and the mean values of the two periods following the wash-out period (experimental values). The excreted quantities of sodium, potassium, osmotically active substances, water and urea were related to the inulin clearance of the corresponding kidney. The urinary concentrations of electrolytes were related to the plasma concentrations as (U/P)-values. The statistical significance of the difference between the normal and affected kidneys for the functions involved was estimated by Student's *t*-test for paired samples.

RESULTS

The protocol of a typical experiment is given in Table II.

The findings in the patients with pyelonephritis are given in Table III. The serum concentrations of sodium, potassium, chloride and urea and the serum osmolality did not change significantly during the experiment; neither was there a significant difference between the inulin clearance (C_{In}) of the two control periods, nor between the C_{In} of the two experimental periods. The BP did not fall after the injection of furosemide.

The values for C_{In} given in Table III are not corrected for BSA. When corrected for BSA the C_{In} of the control kidneys of the patients with pyelonephritis (patients 1–9) in the control period were 65 37 36, 62, 32, 40, 69 51 and 71 ml/min (average 51 4 ml/min). The low normal values in some of the control kidneys may suggest that also these kidneys were affected. The presence of minor pyelonephritic foci in the control kidney which cannot be excluded in any study of this kind, would, however almost certainly have been of no importance to the present study in which the experimental kidneys were severely affected, in addition the functional differences between a normal and a pyelonephritic kidney and between two kidneys affected by pyelonephritis to a different degree are the same in water diuresis (36). It should be added that it has been our experience that the inulin clearance of kidneys supposed to be normal tends to be less when measured by the split function technique with the patient in the lithotomy position than in clearance measurements in the supine position.

Before the injection of furosemide the fractional

U_{osm} (mosm/kg H_2O)		U_{Ur} (mg %)		U_{In} (mg %)		$\frac{C_{H_2O}}{C_{In}}$ (%)		$\frac{C_{osm}}{C_{In}}$ (%)		EF–Na (%)		EF–Cl ( )		V_K/C_{In} (μEq/ml)	
D	N	D	N	D	N	D	N	D	N	D	N	D	N	D	N
74	64	81	108	168	212	11 7	9.7	3.9	2.7	2.8	1 7	2.4	1.3	0.42	0.29
75	63	99	72	161	215	13 9	10 8	4.6	3 0	3.6	1 9	3.2	1.9	0 47	0.32
73	119	89	110	158	162	14.1	10 9	6 9	7 4	3.7	6 5	3.9	2.2	0.50	0.56
183	219	36	50	92	102	11.5	7.0	19 1	20.5	19 1	21.2	21.6	23 6	1.28	1 41
204	205	35	72	99	107	8.4	7.8	19.1	18.1	19 4	18.1	21.8	21.8	1.25	1.19

Table III. *Findings in patients with pyelonephritis, carcinoma and hydronephrosis*

C = control period, E = experimental period, P = statistical probability as percentage, D_C = diseased kidney in control period, D_E = diseased kidneys in experimental period, N_C = normal kidney in control period, N_E = normal kidney in experimental period.

Pat. no.	Period	C_{in} (ml/min) D	C_{in} (ml/min) N	C_{PAH} (ml/min) D	C_{PAH} (ml/min) N	(D/C_{in}) (%) D	(D/C_{in}) (%) N	(C_{osm}/C_{in}) (%) D	(C_{osm}/C_{in}) (%) N	(C_{H_2O}/C_{in}) (%) D	(C_{H_2O}/C_{in}) (%) N	EF Na (%) D	EF Na (%) N	EF-Cl (%) D	EF-Cl (%) N
Unilateral pyelonephritis															
1	C	19.0	70.5	63	253	19.0	9.0	11.7	6.1	7.1	2.8	11.2	4.6	11.8	5.1
2	C	34.8	35.3	201	205	7.5	5.6	3.7	2.0	3.7	3.6	2.3	1.8	2.6	1.8
3	C	42.0	31.5*	140	109	18.0	16.8	10.2	9.2	7.8	7.6	10.5	8.9	11.7	9.7
4	C	27.3	56.3	256	344	11.4	11.8	2.3	1.8	9.1	9.9	1.2	0.7	2.0	1.1
5	C	22.5	30.5	178	212	12.8	9.1	6.1	5.5	6.7	3.6	4.0	3.2	5.2	4.1
6	C	23.5	37.0	132	174	9.7	8.6	7.4	6.5	2.3	2.0	6.0	5.1	6.6	5.6
7	C	5.9	58.5	32	266	17.1	13.1	4.3	2.8	12.8	10.2	3.2	1.8	2.8	1.6
8	C	36.5	42.5	163	172	23.6	21.2	13.7	11.7	9.9	9.5	11.0	9.5	12.4	10.5
9	C	38.0	74.0	187	336	4.7	3.9	5.6	5.3	—	—	4.4	4.1	4.6	4.4
Average		27.72	48.46	150.33	230.11	13.76	11.01	7.23	5.67	7.43	6.15	5.98	4.42	6.64	4.88
$P_{(D_C\ N_C)}$		2.5		3.3		2.8		2.1		NS		4.2		.5	
1	E	19.0	43.5	82	204	34.7	25.3	27.7	19.8	7.0	5.5	29.3	19.8	35.2	25.1
2	E	44.2	33.4	230	179	34.6	24.5	30.2	18.3	4.4	6.1	31.2	21.9	40.0	28.8
3	E	45.5	31.0	144	121	26.8	27.7	20.6	20.8	6.1	7.0	21.2	21.7	26.9	27.0
4	E	59.0	62.0	585	481	15.3	28.0	10.5	20.5	4.7	7.4	9.6	19.9	10.0	27.8
5	E	24.0	20.0	241	172	52.8	54.2	45.6	47.0	7.2	7.2	43.1	44.3	59.6	59.7
6	E	36.0	34.0	172	172	29.9	28.0	28.0	26.0	1.9	1.9	27.2	24.4	36.6	33.7
7	E	8.2	58.5	29	183	29.0	26.7	19.1	19.3	10.0	7.4	19.2	19.7	21.7	22.7
8	E	35.5	37.5	178	197	31.9	30.4	26.3	24.5	5.6	5.9	24.9	24.4	31.8	31.0
9	E	38.5	69.5	212	299	15.2	13.0	15.7	13.5	—	—	14.2	13.0	19.1	16.9
Average		34.43	43.27	208.17	223.17	30.02	28.64	24.86	23.30	5.87	6.05	24.43	23.23	31.21	30.30
$P_{(D_E\ N_E)}$		NS		NS		NS		NS		NS		NS		NS	
$P_{(D_C-D_E)}$		NS		NS		0.3		0.1		NS		0.1		0.1	
$P_{(N_C\ N_E)}$		NS		NS		0.1		0.1		NS		0.1		0.1	
$P\left(\frac{D_C}{D_E}\ \frac{N_C}{N_E}\right)$		0.6		1.4		5.3		NS		3.9		NS		NS	
$P_{(D_C\ D_E\ N_C\ N_E)}$		0.5		1.3		NS		NS		0.8		NS		NS	
Unilateral carcinoma															
10	C	37	49	186	265	8.2	8.6	4.9	4.8	3.5	4.9	4.2	4.7	4.6	4.6
	E	34	48	188	210	23.6	20.1	18.2	15.7	5.4	4.8	18.6	17.0	24.0	22.7
Unilateral hydronephrosis															
11	C	50	49	211	216	16.0	17.4	8.5	9.2	7.5	8.2	5.5	5.9	6.4	9.1
	E	38	38	172	165	17.8	23.6	13.2	18.2	4.8	5.5	11.5	16.8	15.3	20.9

* Small infarct in upper pole.

excretion of sodium (EF–Na) and chloride (EF–Cl) as well as the diuresis, the free water clearance and the osmotic clearance per unit of glomerular filtrate (D/C_{in}, C_{H_2O}/C_{in}, C_{osm}/C_{in}), were greatest in the affected kidneys. The urinary concentration of inulin (U_{in}) was least, in the affected kidneys. This pattern is typical of unilateral pyelonephritis of the human kidney in water diuresis (36, 37) and is also seen in unilateral, experimental renal infection in the dog (12). In correspondance with our earlier findings (36, 37) the urinary concentrations of sodium (and chloride) and the urinary osmolality were not significantly different in the affected and the normal kidneys; neither was the difference between the excretion of sodium (or chloride) in the normal and affected kidneys significant when the excreted fraction was related to the osmotic clearance per unit of glomerular filtrate ((EF–Na/(C_{osm}/C_{in}); (EF–Cl/(C_{osm}/C_{in})), ($p > 0.10$). This indicates that the fractional excretion of sodium and chloride was the same in the pyelonephritic

V_K/C_{In} (µEq/ml)		C_{Ur}/C_{In} (%)		FF		$(U/P)_{Na}$		$(U/P)_{Cl}$		$(U/P)_K$		$(U/P)_{In}$	
D	N	D	N	D	N	D	N	D	N	D	N	D	N
1.32	0.84	71.7	70.0	0.30	0.28	0.59	0.52	0.77	0.57	1.35	1.82	5..4	11.17
0.46	0.51	40.9	44.9	0.17	0.17	0.32	0.35	0.37	0.34	1.68	2.49	13.96	18.89
0.87	0.99	62.0	47.5	0.30	0.29	0.59	0.52	0.65	0.57	1.50	1.23	6.40	5.55
0.74	0.76	69.2	76.6	0.11	0.16	0.11	0.06	0.17	0.09	1.40	1.42	8.85	8.61
1.81	1.79	74.7	98.8	0.13	0.15	0.28	0.35	0.41	0.45	3.28	4.80	7.79	10.96
0.64	0.56	64.0	57.0	0.18	0.21	0.62	0.59	0.68	0.65	1.89	1.87	10.47	11.50
0.45	0.31	52.9	39.8	0.18	0.20	0.19	0.14	0.17	0.13	0.82	0.55	5.90	7.65
1.11	1.00	51.6	60.8	0.22	0.25	0.46	0.44	0.51	0.49	0.94	0.96	4.27	4.76
0.99	0.99	54.4	50.9	0.20	0.22	0.93	1.06	0.97	1.13	4.86	6.10	21.08	24.37
0.93	0.86	60.16	60.7	0.20	0.21	0.45	0.45	0.52	0.49	1.95	2.34	9.33	11.72
NS		NS		NS		NS		NS		NS		2.3	
2.94	2.57	79.8	91.6	0.23	0.21	0.84	0.78	1.08	0.99	1.86	2..4	2.87	3.96
2.88	1.65	114.2	127.6	0.24	0.19	0.90	0.90	1.15	1.17	1.21	1.73	2.83	4.09
1.66	1.80	65.0	69.5	0.32	0.26	0.79	0.78	1.00	0.98	1.76	1.70	3.60	3.83
1.27	2.23	55.6	93.2	0.10	0.13	0.63	0.71	0.66	1.00	1.70	1.72	6.62	3.36
5.22	5.90	134.4	99.5	0.10	0.12	0.81	0.81	1.10	1.10	2.41	2.49	1.87	1.87
1.77	1.74	74.0	64.5	0.21	0.20	0.91	0.87	1.22	1.20	1.69	1.77	3.40	3.59
1.27	1.30	35.0	55.0	0.30	0.32	0.67	0.73	0.88	0.90	1.04	1.16	3.44	3.77
2.07	1.99	56.1	53.4	0.20	0.20	0.79	0.80	0.99	1.01	1.30	1.32	3.19	3.42
2.44	2.39	69.5	46.5	0.18	0.23	0.93	1.05	1.23	1.30	3.73	3.97	5.68	8.34
2.39	2.35	75.96	77.87	0.21	0.21	0.81	0.83	1.04	1.07	1.86	2.01	3.82	4.03
NS		NS		NS		NS		NS		4.1		NS	
0.2		NS		NS		0.1		0.1		NS		0.7	
0.1		NS		NS		0.1		0.1		NS		0.4	
NS		NS		NS		NS		NS		NS		NS	
NS		NS		NS		NS		NS		NS		0.9	
0.6	0.6	63	65	0.20	0.19	0.51	0.50	0.52	0.49	1.98	1.79	12.0	10.5
1.6	1.4	64	58	0.17	0.23	0.80	0.82	1.03	1.07	1.75	1.79	4.2	5.1
1.5	1.6	102	90	0.23	0.23	0.35	0.34	0.40	0.38	2.38	2.27	6.29	5.75
2.1	2.2	94	117	0.22	0.19	0.60	0.72	0.83	0.89	2.97	2.28	5.46	4.28

and normal kidneys at identical excretion rates of osmotically active substances per ml of glomerular filtrate.

The relation between the excretion of urea and inulin (C_{Ur}/C_{In}) and the filtration fraction (FF) was not significantly different in the affected and the normal kidneys.

The excretion of potassium per unit of glomerular filtrate (V_K/C_{In}) was not significantly different in the affected and the normal kidneys. The urinary concentration of potassium was less in the diseased than in the normal kidneys, but the difference was not statistically significant.

After the injection of furosemide the fractional excretion of sodium increased to 23 (13 to 44)% of the filtered load and the diuresis increased to 28 (13 to 54)% of the glomerular filtrate. Thus the effect of furosemide was about maximal, and of a magnitude similar to that observed in experiments on humans and animals in which far greater doses of furosemide have been used (5 6 44 48 49).

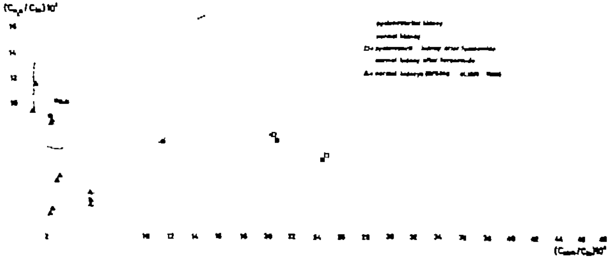


Fig 1 Free water clearance and osmotic clearance in normal and diseased kidneys. Stippled area at left. data from normal kidneys of dog and man in water diuresis. Stippled area in the middle: data from normal dogs in hypotonic saline diuresis after increasing the osmotic clearance by pretreatment with DOCA. Stippled area at right: data from normal dogs in hypotonic saline diuresis after injection f furosemide. Triangles: normal kidneys of patients with unilateral renal disease without injection of furosemide.

The clearance of inulin and *p*-aminohippuric acid increased in the pyelonephritic and decreased in the normal kidneys after the injection. The differences between the control values and the experimental values were significantly greater in the diseased than in the normal kidneys. As the extraction ratio of *p*-aminohippuric acid (*E*–PAH) is not influenced, or only temporarily slightly re duced, by furosemide (6, 38), the variations in the *p*-aminohippuric acid clearance indicate cor responding variations of the renal blood flow The filtration fraction and the relation between the excretion of urea and inulin (C_{Ur+}/C_{In}) were not significantly influenced by the furosemide injection although, as expected (13) and in ac cordance with the findings of others (42) the ratio (C_{Ur+}/C_{In}) increased in most patients when the diuresis increased.

The excretion and the urinary concentrations of sodium, chloride and osmotically active substances increased to the same extent in the nor mal and the diseased kidneys. After the injection the differences between the EF–Na, EF–Cl, D/C_{In}, C_{osm}/C_{In}, C_{H_2O}/C_{In} and U_{In} of the diseased and the normal kidneys, although still present, were no longer significant. The relation between the sodium excretion (or chloride excre tion) and the osmotic clearance ((EF–Na/(C_{osm}/C_{In})· (EF–Cl/(C_{osm}/C_{In})) of the diseased and the normal kidneys increased after the furosemide injection ($p < 0.01$), but as in the control periods, there was no significant difference between these relations in the diseased and the normal kidneys ($p > 0.10$).

The excretion of potassium also increased, but without a significant increase of the potassium concentration in the urine. The urinary concentration of potassium was less in the diseased than in the normal kidneys after furosemide ($p <$ 0 05)

With the exception of patient 6, *the free water clearance* increased in kidneys with low control values and decreased in kidneys with high free water clearance in the control periods. In Fig. 1 the free water clearance has been correlated with the osmotic clearance of the same individual earlier data of normal human kidneys investigated by an identical technique, but without injection of furosemide (35 37), and data from an investigation of nonanesthetized dogs (43) have been included. When compared with the kidney function of non-injected individuals in water diuresis, the free water clearance at corresponding osmotic clearance was reduced in all kidneys af ter the injection of furosemide. The difference between the free water clearance per unit of glomerular filtrate in the diseased and the normal kidneys was not significant. In both the diseased and the normal kidneys there seemed to be a maximal value of about 7 ml/min for the free

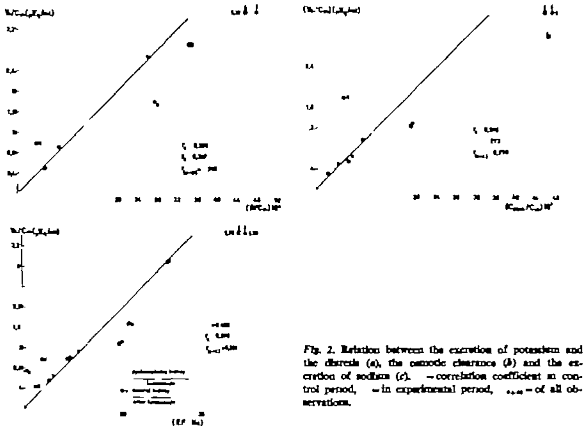


Fig. 2. Relation between the excretion of potassium and the diuresis (*a*), the osmotic clearance (*b*) and the excretion of sodium (*c*). — correlation coefficient in control period, — in experimental period, — of all observations.

water clearance per 100 ml of glomerular filtrate after the furosemide injection. Similar findings have been reported by Suki et al. (48) in unanesthetized dogs.

The relation between the excretion of potassium and the excretion of sodium, the osmotic clearance and the diuresis are given in Fig. 2. The excretion of potassium showed weak, positive correlations with the excretion of sodium (EF–Na), the osmotic clearance (C_{osm}/C_{In}) and the diuresis (D/C_{In}). The relation between the excretion of potassium and the excretion of sodium (EF–Na/(V_K/C_{In})) was significantly greater in the affected than in the non-affected kidneys in the control periods ($p < 0.02$), and unlike the findings in the dog (48) this ratio increased significantly after the injection ($p < 0.01$). After the injection there was no longer a significant difference between the ratio of the diseased and the normal kidneys ($p > 0.01$).

There was no significant difference between the ratio of the excretion of potassium and the diuresis ((V_K/C_{In})/D/C_{In}) in the diseased and the normal kidneys either in the control period or in the experimental period, and this relation did not change significantly either in the diseased or in the normal kidney after the injection of furosemide ($p > 0.01$).

In the patient with unilateral reduction of the renal mass due to a *renal carcinoma* the functional pattern of the diseased and the normal kidney was the same before and after the injection of furosemide, and the effect of furosemide was similar in both kidneys.

In the patient with *unilateral severe hydronephrosis* the excretion of water sodium, chloride, the free water clearance and the osmotic clearance were slightly less in the hydronephrotic than in the normal kidney: this pattern of nephron hypoperfusion has also been observed in acute hydronephrosis in the dog (47). These differences increased after the injection of furosemide. In this patient the effect of furosemide upon the diuresis of the pyelonephritic kidney was negli-

gible in the normal kidney the diuresis increased 36%. The effect of furosemide upon the EF–Na and the urinary concentrations of sodium and chloride in the hydronephrotic kidney although less than in the normal kidney were of the same order of magnitude as in the other kidneys in this investigation. This combination of a constant diuresis per unit of glomerular filtrate and an increase of the urinary electrolyte concentrations was not observed in any other kidney

DISCUSSION

As the main effect of furosemide is restricted to the loop of Henle, the almost identical effect of furosemide upon the fractional excretion of sodium and water in the normal and the diseased kidneys indicates that nephrons with intact loops were present in the diseased kidneys, and might suggest that a normally functioning loop was present in most of the functioning nephrons in the diseased kidneys. The finding of an identical maximal value for the free water clearance per 100 ml of glomerular filtrate in normal and diseased kidneys after the injection also suggests that the functioning nephrons in the diseased kidneys had normally functioning loops of Henle, as this loop is part of the diluting segment of the nephron.

A recent argument against the hypothesis of intact nephrons has been based upon the observations that rat kidneys with different types of experimentally induced toxic necrosis of the proximal tubule, the loop of Henle being left intact, are able to imitate some of the functions of the normal kidney the reduced reabsorption of sodium in the proximal tubule being partially compensated for by increased reabsorption in the distal parts of the nephron (7). The idea of irregularly distributed functionless nephron segments alternating with normal or hypertrophic segments which compensate for the functional defects cannot, however be applied to all the findings in the present study The production of free water is a specific function of the diluting segment; therefore, as far as the dilution process is concerned, necrosis of this segment cannot be compensated for by other parts of the nephron.

The quantity of free water excreted is influenced by the function of both the proximal and the distal tubule. However the pari passu variations of the free water clearance per unit of glomerular filtrate in the diseased and the normal kidneys, as well as the increase of the free water clearance in some patients and the decrease in others toward a common maximal value, without relation to concomitant changes of the glomerular filtration rate, seem to be best explained by a normal effect of furosemide upon normally functioning loops of Henle. This interpretation is compatible with the observation that the net transport of water out of the tubular lumen, a function which is also dependent on an intact loop of Henle, has been shown to be present in diseased kidneys of dogs and humans, and in some instances even to be almost normal (3 12, 16)

It must be added that, although the main effect of furosemide seems to be restricted to the loop of Henle, the sodium transport in the proximal tubules is also reduced, but probably without substantial decrease of the fractional reabsorption (5 9 15 25 32, 41 48) In addition to this, furosemide increases the intrarenal pressure (21 26) and also affects the magnitude (49) and possibly also the distribution (8) of the renal blood flow The almost identical function in normal and diseased kidneys after this complex stimulus might be taken as a point in favour of an identical effector site, the nephron, in the diseased and the normal kidneys.

The identical and great increase of the tubular flow in normal and diseased kidneys suggests that also the anatomy of functioning collecting ducts was not far from being normal in the diseased kidneys, as the greater part of the flow resistance in the tubular system of the kidneys seems to be localized to these structures both under normal conditions as well as after injection of furosemide (8, 20 ..1).

The findings in the hydronephrotic kidney in patient 11 in which furosemide increased the excretion fraction of sodium without increasing the diuresis, probably demonstrated the importance of normal collecting duct and renal pelvic anatomy when the tubular flow is increased to very high levels.

The findings in the patient with loss of parenchyma due to a renal cell carcinoma indicate that the loss of tissue per se did not influence the effect of the furosemide injection.

A fundamental point in the hypothesis of intact nephrons has been that the functional dif

ferences between diseased and normal kidneys, for example those observed in the control periods in the present study are due to a greater glomerular filtration rate per nephron in the diseased kidney (4 10 11 12, 31) The disappearance of these differences in the experimental periods in the present study can, however not be explained by an absolute or relative reduction of the glomerular filtration rate in the diseased kidneys after the injection of furosemide. On the contrary the glomerular filtration rate increased in the diseased and decreased in the normal kidney in most patients.

The reabsorption of sodium and water is in part dependent upon the colloid osmotic pressure in the peritubular capillaries, which again is a function of the filtration fraction (19 29). The filtration fractions in the pyelonephritic and the normal kidneys were not significantly different and did not change after the injection of furosemide. Consequently there is no evidence that the findings concerning the fractional excretion of sodium and water before and after furosemide were caused by a difference in colloid osmotic pressure in peritubular capillaries of the normal and the diseased kidneys.

The reabsorption of sodium and water is also influenced by the intrarenal pressure (18, 19 33). Recent studies (30) indicate that the increased excretion fractions of sodium and water in hypertensive patients, and also in normals, following an infusion of saline are correlated with an increase of the intrarenal pressure. There is therefore a theoretical possibility that the greater rejection fraction of sodium in the pyelonephritic kidneys in the control periods was caused by a greater intrarenal pressure in the pyelonephritic than in the normal kidney and that this difference in function disappeared or became reduced when the intrarenal pressure in both kidneys increased, following the injection of furosemide (21 26). The intrarenal pressure in pyelonephritis in man is, however unknown.

A possible difference in function between the kidneys after the injection of furosemide might have been disguised by the wash-out effect. Due to the great diuresis in the control period the expected wash-out effect was small and was probably eliminated by the exclusion of the "wash-out period" following the injection of furosemide from the calculations. In the case of urea the wash-out effect would have manifested itself by diffusion of urea from the medulla into the collecting ducts during the transition from water diuresis to the furosemide-induced diuresis (1 2, 22, 28, 51). The finding of a greater clearance of urea than of inulin in the experimental periods in 3 out of the 18 kidneys investigated might indicate a wash-out effect in these kidneys; however the ratio between the clearances being based upon composite measurements, the differences observed were probably not significant. The average rise of the ratio between the urea and the inulin clearance was not significant; neither was a significant difference between the inulin clearance of the first and second experimental period observed.

The excretion of potassium in unilateral renal disease in man seems not to have been described before. Unlike the excretion of sodium, the excretion of potassium per unit of glomerular filtrate was not significantly different in diseased and normal kidneys, and the increased potassium excretion after furosemide seemed to be only a secondary effect of the increased diuresis. This might suggest that the functional differences between the diseased and normal kidneys were established in other parts of the nephron than where the potassium excretion takes place, i.e. in the proximal tubule and the loop of Henle. A well-preserved function of the distal tubule is also compatible with the fact that the excretion of acid and the ability to establish an H -gradient between the tubular cells and the tubular lumen is usually normal (17 46) in diseased nephrons, although there are exceptions (53) The finding of a lower potassium concentration in the urine after the injection of furosemide, however suggests some functional derangement also in the distal portion of the nephron in the diseased kidneys.

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POST-GAMMA PROTEIN IN URINARY PROTEIN

Occurrence, Clinical Significance and Probable Identity with Muramidase

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Abstract. In an examination of 3 200 urinary protein postgamma fraction has been found in 13 patients. These patients constituted two groups, one with leukemias of the myeloid (acute or chronic) or monocytic types (8 patients), and one with nephropathies (5 patients). Eight patients were reexamined with simultaneous urinary protein paper electrophoresis and urinary muramidase (lysozyme) determinations. In four of these patients both post-gamma protein and elevated urinary muramidase activity were found, muramidase activity being localized to the post-gamma fraction. In the remaining four patients neither post-gamma protein nor grossly elevated muramidase activity could be demonstrated (probably attributable to treatment). The appearance of post-gamma protein in urinary protein paper electrophoresis is thus a rare occurrence, should prompt examination for muramidase activity and may indicate either leukemia of the myeloid or monocytic type and/or nephropathy.

In 1961 Butler and Flynn (1) reported the finding of a protein with post-gamma mobility in the urine from certain patients, especially patients with renal tubular defects such as the Fanconi syndrome. The protein was not identified. In 1966 Osserman and Lawlor (5) reported on a post-gamma protein found in the urine of patients with monocytic leukemia. These authors identified the protein in their patients as muramidase.

The aim of the present work was, in a large material of urinary protein paper electrophoreses, to examine the frequency and clinical significance of the post-gamma protein and to investigate whether or not this protein could be identified as muramidase.

MATERIAL AND METHODS

In the period 1957–1970, 3 200 urinary protein paper electrophoreses were carried out at the Protein Laboratory of the University of Copenhagen. The main reason for referring patients was proteinuria. Protein electrophoresis on urine was performed by the method of Tiselius (7, 8). These 3 200 paper electrophoresis strips were examined for the presence of a post-gamma fraction, i.e. a protein fraction on the cathodic side of the gamma globulin, clearly distinct from the latter (Fig. 1).

Patients with post-gamma protein who were still alive at the time of the present study were examined with simultaneous urinary paper electrophoresis and muramidase determinations in plasma and urine. In order to localize muramidase activity the paper strips were cut into about 40 pieces (Fig. 1). Each piece was eluted in 2 ml of the veronal buffer used for electrophoresis, and muramidase activity was determined in each of the fractions.

Muramidase activity was measured with the lyso-plate method of Osserman and Lawlor (5) with hen egg white as a standard. Normal values for plasma muramidase activity in our laboratory are 104 ± 53 mg/l (1 S.D.) (2), and for urinary muramidase activity below 4.40 mg/l. It should be noted that with hen egg white as a reference standard muramidase activities are about ten times higher than with human muramidase as a reference (5).

RESULTS

Of the 3 200 paper electrophoretic runs 13 showed a post-gamma protein as described above.

Table I summarizes the diagnoses and relevant clinical information, especially concerning hematological and renal status of these patients. It is seen that the patients could be divided into two groups, one with leukemias of the granulocytic or monocytic types and one with renal disorders.

Of the 13 patients five had died before the present study. In one of these patients (no. 8) plasma muramidase activity was measured; plasma had been kept in a deep freezer for one year before analysis. The remaining patients were ex-

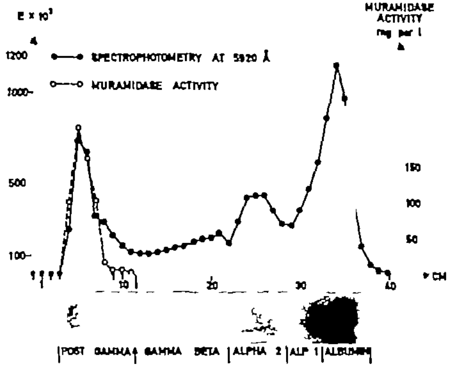


Fig. 1. Urinary protein paper electrophoresis and muramidase activity showing post-gamma fraction and localization of muramidase activity.

amined with urinary protein paper electrophoresis and tested for urinary muramidase activity on the same sample of urine. Table II summarizes the results of these examinations. It is seen that of the eight patients four (nos. 3, 6, 7 and 13) had both a post-gamma fraction and increased urinary muramidase activity. Patient 8 had extremely high plasma muramidase activity well above the supposed renal threshold (3); consequently this patient most likely would have had increased urinary muramidase activity also. Four patients had neither a post-gamma fraction in the paper electrophoresis nor grossly increased urinary muramidase activity.

In three patients with concomitant post-gamma protein and muramidasuria, muramidase activity was localized in the post-gamma fraction with no activity in the other protein fractions (Fig. 1). One patient (no. 7) had muramidase activity in a small band in the gamma fraction in addition to post-gamma activity. Regression analysis on the correlation between the concentration of post-gamma protein and muramidase activity (Table II) showed a correlation coefficient of 0.99.

DISCUSSION

In a series of 223 patients Butler and Flynn (1) found by starch gel urinary protein electrophoresis a post-gamma fraction in 46 patients, including 19 with the Fanconi syndrome and 9 with multiple myeloma. The much higher incidence of post-gamma protein in that series than in ours might in part be explained by a difference in method, as starch gel electrophoresis is a more sensitive method than the paper electrophoresis used in this study.

From Table II it appears that the level of urinary muramidase activity was between 33 and 140 mg/l before the post-gamma fraction could be discovered by paper electrophoresis. This fact indicates that paper electrophoresis is a rather insensitive method to reveal muramidasuria, and, when the post-gamma fraction appears, it indicates a severe abnormality if identity with muramidase can be established.

The identity of the post-gamma protein with muramidase is supported by the fact that in four patients muramidase activity was shown to be localized in the post-gamma fraction. Also the

Table I. *Clinical data on 13 patients with urinary post-gamma protein*

CML – Chronic myeloid leukemia. AML – Acute myeloid leukemia. AMol – Acute monocytic leukemia

Pat. no.	Sex	Age (y.)	Diagnosis	Leuko-cytes (per µl)	Differential	Serum creatinine (mg/100 ml)	Renal histology	Post gamma protein (mg/l)
1 (1969)	♀	82	CML	316 000	Dominated by immature neutrophils	1.9	—	8
2 (1967)	♀	64	CML	59 100	Dominated by immature neutrophils	1.1	—	56
3 (1969)	♀	29	CML	442 000	Dominated by immature neutrophils	1.0	—	290
4 (1969)	♀	68	CML	22 400	Dominated by immature neutrophils	8.7	Leukemic infiltrat.	18
5 (1966)	♂	10	AML	201 000	99 % myeloblasts	1.0	Leukemic infiltrat.	33
6 (1969)	♂	38	AMol	12 900	77 % monoblasts	0.8	—	73
7 (1969)	♂	82	AML	9 000	83 % myeloblasts	1.2	—	3
8 (1968)	♂	69	AML Multiple myeloma	4 300	13 % myeloblasts	1.6	Tubular atrophia Thickening of glomerular base membrane	240
9 (1968)	♀	43	Systemic lupus erythematosus	6 200	Normal	1.1	Lupus nephritis. No tubular damage	207
10 (1966)	♀	39	Uremia. Chronic pyelonephritis Malignant melanoma	9 000	Normal	12.0	Chronic pyelo-nephritis	3
11 (1968)	♀	58	Collagen disease Hypercalcemia	19 900	37 % eosinophils, otherwise normal	1.9	Atrophic tubules	1/2
12 (1968)	♂	55	Idiopathic nephrocalcinosis	10 600	Normal	1.1	—	5
13 (1965)	♂	58	Benign parathyroidoma. Idiopathic nephropathia	4 200	Normal	1.6	—	2

high correlation in the regression analysis between post-gamma protein and muramidase activity is in favour of the identity. The lower values of the post-gamma concentrations compared to the muramidase activities are probably due largely to loss of muramidase during concentration of the urine. Thus muramidase activity was shown to be present outside the dialysis bag after pressure dialysis (cf. the low molecular weight of muramidase). Also it is unknown whether the staining of the paper strips gives a fair estimate of the post-gamma protein.

Furthermore, although not proven, the identity of the post-gamma protein with muramidase in the remaining patients is supported by indirect evidence. Thus these patients belonged to the same categories of diseases, and when the post gamma protein had disappeared, probably as a result of treatment, no significant muramidasuria was found either

In the present study the patients with urinary post-gamma protein constitute two groups, one with leukemias (8 patients), and one with renal disorders (5 patients).

The patients with leukemia were all of the myeloid and monocytic types (acute and chronic), which agrees well with the contention that the post-gamma protein in these patients was identical with muramidase. It is well known that plasma and urinary muramidase activities decrease with successful treatment (i.e. destruction of muramidase containing leukemic cells) (6) thus explaining the disappearance of the post-gamma fraction in some of our patients at reexammation.

Table II. *Clinical data and simultaneous determinations of urinary post-gamma protein and muramidase activity in urine and plasma*

Pat. no.	Leukocytes (per μl)	Differential	Serum creatinine (mg/100 ml)	Post-gamma protein (mg/l)	Urinary muramidase (mg/l)	Plasma muramidase (mg/l)
1 (1969)	24 800	Dominated by immature neutrophils	1.2	0	33	330
2 (1969)	14 900	Dominated by immature neutrophils	0.8	0	5	68
3 (1969)	442 000	Dominated by immature neutrophils	1.0	290	7 200	1 270
6 (1969)	12 900	77% monoblasts	0.8	75	1 260	—
7 (1969)	8 900	83% myeloblasts	1.2	10	405	—
8 (1968)	4 300	13% myeloblasts	1.6	—	—	655
9 (1969)	7 400	Normal	1.0	0	3	38
11 (1969)	11 900	20% eosinophils	1.4	0	0	88
13 (1969)	7 100	Normal	2.1	1.3	140	125

The non-leukemic patients of this study constitute a rather heterogeneous group. Although the mechanism for the excretion of muramidase in these patients cannot be exactly stated, it seems reasonable to attribute it to the common denominator of these patients, namely the impaired renal function. Muramidase is filtered in the glomeruli and reabsorbed and probably catabolized in the proximal tubules. Thus muramidasuria is found in uremia with the increased load of muramidase imposed on the remaining hyperfunctioning nephrons, and in patients with renal tubular defects. Of our patients one (no. 10) had pronounced uremia, and two (nos. 11 and 12) had diseases associated with tubular defects. The excretion of post-gamma protein in patient 9 with lupus nephritis, normal glomerular function as judged by the serum creatinine and no tubular damage at biopsy is difficult to explain on the basis of our present knowledge but is in agreement with the findings of others (3). The kidney disease of patient 13 was unexplained. Finally it should be recognized that in the patients where muramidase was not demonstrated in the post-gamma fraction the possibility that the post-gamma protein was identical with the gamma-trace protein (4) cannot be excluded.

ACKNOWLEDGEMENTS

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COAGULATION AND FIBRINOLYSIS IN YOUNG FEMALE ARTERITIS

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Abstract. Extensive and repeated analysis of the coagulation and fibrinolytic systems in five women with young female arteritis revealed normal content of coagulation and fibrinolytic factors in the circulating blood, normal platelet count and platelet adhesiveness in all five, poor fibrinolytic response to venous occlusion and/or reduced plasminogen activator content in superficial vessel walls of four patients studied. In spite of the limited number of patients the findings suggest defect of the fibrinolytic system, which may contribute to the eventual development of arterial thrombosis.

The aetiology of young female arteritis is still unknown. However circulating antiarterial antibodies have been demonstrated in patients with this disease (9, 13, 28) and autoimmunisation has therefore been considered a possible cause. But it is still debatable whether the antibodies demonstrated are due to an altered immunological response or to degeneration of the arterial tissue (14). An abnormally high platelet count and increased plasma fibrinogen have also been reported (1, 2, 23, 30). These changes have been regarded as signs of hypercoagulability inviting deposition of fibrin on the arterial wall with secondary injury to the vessels involved. Some investigators (4, 6, 8, 10, 14) have been unable to confirm such an increase in the platelet count or the fibrinogen. In fibrinolytic studies Atsumi (?) found the euglobulin lysis time to be prolonged and the antiplasmin titre to be increased in some moderate and severe cases (groups 2 and 3 according to Inada (10) and Nakao (14)).

This paper concerns the various components of the coagulation and fibrinolytic systems in young female arteritis with special reference to the response of the fibrinolytic activity to venous occlusion as well as the plasminogen activator content of the wall of superficial veins.

MATERIAL AND METHODS

The series consisted of five women, aged 17 to 25 at the time of appearance of the disease, with angiographically verified stenosis and/or occlusion of the major branches of the aortic arch (Table I). Examination had also revealed one or more of the following abnormalities in the arm: non-palpable pulse, low blood pressure or low plethysmographic values. All patients showed symptoms of rheumatic disease (muscle pain, languor and elevated ESR) at some time during the disease.

Collection of blood. The blood, collected by the silicone technique, and citrated plasma and serum were prepared in the way described previously (19, 27).

Coagulation studies. The following determinations were made: platelet count, platelet adhesiveness in whole blood, coagulation time in glass and plastic tubes, one-stage prothrombin time, recalcification time of plasma, prothrombin + factor VII + factor X (Owren, P & P-test), factor V, factor VIII, factor IX, fibrinogen and thrombin time. Unless otherwise stated, the methods described previously (18) were used for the determinations. The platelet adhesiveness was measured by the method of Hellem (7) as modified by Cronberg et al. (5). The thrombin time was determined in the way described by Nilléhn and Nilsson (16), the plasma fibrinogen as described by Nilsson and Olow (20). Only fibrinogen values for blood collected with E-ACA are given.

Fibrinolytic studies. The following determinations were made: fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated fibrin plates, plasminogen (clot method), inhibitors of plasminogen activation by urokinase (urokinase inhibitors), antiplasmin (α_2AT) and fibrinolytic split products (15, 17, 21, 22, 27).

Venous occlusion was produced by placing a sphygmomanometer cuff around the upper arm or thigh and inflating to a pressure midway between the systolic and diastolic blood pressures in the limb for 20 min. Blood samples for determination of the fibrinolytic activity were

Table I *Survey of clinical material*

	Case no. 1	2	3	4	5
Age at appearance of disease	18	17	19	25	23
Age at investigation	26	21	36	37	24
"Rheumatic" symptoms at appearance of disease	+	+	+	+	+
Activity of disease at investigation	+	+	−	+	+
α_2-globulin during active disease (g/100 ml)	0.73	0.75		0.65	0.60
γ-globulin during active disease (g/100 ml)	1.52	1.69		1.79	1.10
Affected arteries (angiography)	a. subclavia sin. a. vertebralis sin.	a. subclavia sin.	a. carotis com. dx. a. subclavia dx. a. vertebralis dx. a. renalis dx. aorta abdominalis	a. carotis com. dx. a. subclavia dx. a. subclavia sin. a. vertebralis sin.	a. axillaris dx. a. subclavia sin.
Prednisolone treatment at the time of investigation (mg/day)	5	7.5		5	

drawn before application of the cuff and immediately before the cuff was deflated. The samples from the arms were collected from an antecubital vein; those from the legs, from the distal part of the long saphenous vein.

Assay of the plasminogen activator content in the veins. Biopsy specimens of superficial veins were obtained under local anaesthesia (0.5% Carbocain®) from the distal part of the cephalic vein. The veins were examined by the fibrinolysis autography technique of Todd (31) as modified by Pandolfi et al. (24, 26). The fibrinolytic activity of the veins was estimated in the following way. Four fibrin slides were prepared for each specimen and incubated at

Table II *Components of the coagulation and fibrinolytic systems*

	Case no. 1		2		3	4		5		
	11/66	3/68	11/66	2/67	3/67	4/67	3/68	6/67	12/67	Normal range
Coagulation time (min)										
Glass	9		12	12	13	15	7	12		8–14
Plastic	17		18	22	30	28	28	22		15–25
Platelet count (per mm³)	240 000		300 000	400 000	320 000		320 000	300 000	370 000	200 000–400 000
Platelet adhesiveness (%)	40		32	40	32		38	41	32	22–40
One-stage prothrombin time (sec)	20		17	19	17	18	15	15		15–17
Recalcification time (sec)	127		180	125	113	132	115	165		120–160
Thrombin time (3 NIH U/ml) (sec)	16		21	13	21	23	22	20		18–20
P & P (%)	63*		108	94	84	100	112	73		80–120
Factor V (%)	96		111	109	145	88	100	124		80–120
Factor VIII (%)			100		112	207	88	153		60–160
Factor IX (%)			97			50		42		60–160
Fibrinogen (g/100 ml)	0.37		0.50	0.35	0.36	0.42	0.35	0.41		0.22–0.38
Plasminogen (%)				89		109	97	101		60–140
Urokinase inhibitors (%)	190	76	91	107	108	121	135	115	160	60–140
Antiplasmin (%)			101		87		100	122		60–140
Fibrinolytic split products (mg %)		0	0	0	0	0	0	0		0

* Dicoumarol therapy

Table III. *Fibrinolytic activity before and during venous occlusion as well as in venous biopsy specimens*

– plasma (unheated plates) mm² – resuspended euglobulin precipitate (unheated plates) mm²

	Case no. 1		2			3		4		5		Normal
	11/66	3/68	11/66	2/67	2/68	3/67	2/68	4/67	3/68	6/67	12/67	range
Spontaneous fibrinolytic activity	0	0	0	0	0	39	0	0	0	0	0	0–50
	30	0	33	34	10	53	10	0	0	20	25	0–70
Fibrinolytic activity during venous occlusion												
Right arm	90	71	56	0	93	0	256	235	125	35	25	0–390
	62	49	23	67	117	225	234	240	137	49	22	100–450
Left arm	108	0	56	0		399	256	65	113	32	105	
	132	0	87	27		424	283	269	153	50	79	
Right leg	0	0	20	0	0	167	0	23	0	0	0	0–150
	31	0	20	65	0	105	0	35	61	29	30	10–170
Left leg	0	0	0			111	0	0	0	0		
	43	0	35			88	17	22	23	0		
Fibrinolytic activity of venous biopsies (arbitrary units)		3		8.5[a]		3		6.5[a]		9[a]		6–12

[a] Right arm. [b] Left arm

37°C for 0, 5, 10, 20 min, respectively. Three fairly distinct degrees of fibrin digestion were recognized, namely grade I, microscopical punctate areas of lysis in most of the sections; grade II, gross lytic areas of irregular outline and sometimes confluent; grade III, dissolution of most or all the fibrin in contact with the sections. A grade I slide was allotted 1 point; grade II slide, 2 points; and grade III, 3 points. The total number of points scored by the set of four slides was taken as measure of the fibrinolytic activity of the sample.

RESULTS

No systematic change was found in any of the coagulation factors studied (Table II). The platelet count and platelet adhesiveness were invariably normal. The values found for spontaneous fibrinolytic activity, plasminogen, inhibitors of plasminogen activation by urokinase, antiplasmin and fibrinolytic split products fell within or though only occasionally just outside the normal limits. During the 6–16 month period covered by the investigation repeated venous occlusion of the arms for 20 min produced little or no increase in the fibrinolytic activity in three of the patients (nos. 1, 2 and 5) but a normal increase in the other two (Table III). The increase in fibrinolytic activity during venous occlusion of the legs was within normal limits. The plasminogen activator content in the wall of a superficial vein was low in two of the patients (nos. 1 and 3), but normal in the other three.

DISCUSSION

Young female arteritis has been studied extensively. But only few investigators have focused attention on the coagulation and fibrinolytic systems, besides which their findings are difficult to evaluate because the analytical methods and/or the normal ranges used are not described. Some investigators have described the fibrinogen as increased (1, 2, 23, 30). Paloheimo (23) found that in 22 of his 35 patients the fibrinogen exceeded 420 mg/100 ml, but in only two of them did the high level persist at later control. In none of our patients was the fibrinogen invariably increased. Paloheimo also found that in 11 of 33 patients the platelet count exceeded 350 000/mm³

but that the count was constantly increased in only one. Other authors (2, 3 6, 10 14) have found the platelet count to be normal. We found none of the coagulation factors to be invariably abnormal in any of our patients. Our findings, then, do not lend support to the assumption of an increased clotting tendency as a possible cause of the disease (1 23).

The values found for the components of the fibrinolytic system in the circulating blood always fell within or just outside the normal limits. Atsumi (2) found the fibrinolytic activity in the circulating blood to be decreased. This discrepancy may perhaps be explained by the inadequacy of his control material (6 normals) and, second, by the fact that the most pronounced changes in his patients were found in groups 2 and 3 which were not represented in our material. Besides this a low habitual fibrinolytic activity is difficult to measure with any degree of accuracy unless the patient serves as his own control.

During venous occlusion of a limb the local fibrinolytic activity normally increased (4) and more in the arms than in the legs (25). In control series consisting of 300 apparently healthy persons the increase of the fibrinolytic activity produced by venous occlusion was subnormal in only 15 (29) Of our five patients with young female arteritis three were poor responders. The poor response was not due to treatment with prednisolone, since the response of the fibrinolytic activity to venous occlusion is not influenced by this drug (11). Of the three patients who were poor responders, one had a low plasminogen activator content in the wall of a superficial vessel. The lowness of this value may to some extent be an effect of prednisolone, which reduces the content of plasminogen activator in the vessel wall (11). Moreover one patient who was not treated with prednisolone and who showed a normal local response of the fibrinolytic activity to venous occlusion had a low plasminogen activator content in the vessel wall. This means that four of the patients responded poorly to venous occlusion and/or had a reduced amount of plasminogen activator in the walls of the superficial veins. Of patients with recurrent idiopathic deep venous thrombosis or thrombophlebitis, fibrinolysis was defective in about 73% (12). If we compare these results with the findings in young female arteritis, it appears that the permanent defect in the fibrinolytic system may be a contributory cause of the eventual development of thrombosis in the large and medium-sized arteries (3 8, 23) These investigations suggest the existence of hypofibrinolysis rather than of increased clottability in young female arteritis.

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FIBRINEMIA IN MEDICAL PATIENTS SCREENED BY THE ETHANOL TEST

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Abstract. Thrombin generation leads to the formation of soluble fibrin in plasma. Such plasmas are gelled by ethanol at room temperature. This ethanol test has been performed on 948 consecutive patients admitted to our Department of Internal Medicine, 108 (11.4%) demonstrating gelation of plasma. Re-examination of the positives lowered this incidence to 7.2%. Thus fibrinemia was not rare among these patients. Plasmas from 275 healthy controls, and serum from the patients, were uniformly negative. Using the main diagnosis at discharge, there was a highly significant difference ($p < 0.0005$) in the disease pattern of the patients having a positive and those having a negative test. A preponderance of malignant neoplasms, pneumonias, and diseases of the blood, and blood-vessels, including pulmonary embolism, was found among the patients with positive test. A highly significant increase in number of deaths ($p < 0.0005$) was found among patients with a positive ethanol test. Thus low grade intravascular coagulation may contribute to fatal outcome.

The significance of disseminated intravascular coagulation (DIC) as a pathogenic mechanism in several clinical conditions is becoming increasingly appreciated (11, 20, 21). Massive DIC is usually *readily detectable because of severe depletion of* blood platelets, fibrinogen and other coagulation factors (25, 37). In the absence of frank defibrination, however, platelet count and clotting time analyses frequently offer little help in establishing this diagnosis, since similar results may occur in several disease states, e.g. liver insufficiency, malabsorption, multiple transfusions (13, 24, 28).

Demonstration of soluble fibrin in plasma is a more sensitive and reliable indicator of DIC (33). Soluble fibrin may be demonstrated by various precipitation tests (1, 18, 36) or by gelation of plasma upon the addition of ethanol (8, 19). Several reagents may precipitate proteins from normal plasmas (32). Thus precipitation tests should be regarded as less specific than gelation tests. In Lyons revised gelation test (4) b-naphtol was used. This compound leads to precipitation and renders that test less specific (7). By end group analyses and opacity studies, performed in this laboratory, it was demonstrated that addition of ethanol to plasma prompted rapid gelation when soluble fibrin was present (8). Based on this observation we suggested this ethanol test as a rapid screening test for intravascular coagulation (9). This test has been repeated and confirmed by others (2, 3, 35).

The sensitivity and simplicity of the ethanol test prompted us to examine a large number of persons in order to evaluate: 1) the frequency of a positive ethanol test in healthy volunteers; 2) the frequency of a positive test in unselected patients admitted to our Department of Internal Medicine; 3) in which diseases a positive test was found, 4) if there was a different fatality in patients having a positive test.

MATERIAL AND METHODS

Reagents

Heparin (Leo). 5 000 U/ml.

Hirudin (Sigma). 10 mg was dissolved into 1 ml of saline.

Citrate solution. 3.13 g of sodium citrate dihydrate was dissolved into 100 ml of distilled water.

Heparin/citrate solution. 500 U (0.1 ml) of heparin was added to 9.9 ml of the citrate solution.

Hirudin/citrate solution. 1 mg (0.1 ml) of hirudin was added to 9.9 ml of the citrate solution.

EDTA solution. 2.86 g of disodium ethylenediamine tetraacetic acid, dihydrate, was dissolved in distilled water, pH adjusted to 7.4 with NaOH, and water added to give 100 ml solution.

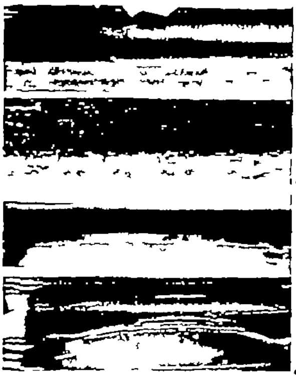


Fig. 1 Results of the ethanol test. (*a*) and (*b*) negative tests, (*c*) and (*d*) positive tests

Ethanol solution. A 96% solution of ethanol (51 ml) was diluted with distilled water (49 ml) to a 50% solution (approx.) and kept at room temperature in [illegible] oppered bottle.

Platelet poor plasma. Blood was collected into polystyrene tubes in the fasting state. Whole blood was obtained with [illegible] minimum of stasis, preferably from an antecubital vein, through an 18 gauge, disposable needle discarding the first few milliletres. Samples from poorly executed punctures were discarded. Nine parts of whole blood were collected into a precooled polystyrene tube containing one part of anticoagulant solution. The contents were thoroughly mixed, and centrifuged within 1 h (during this period kept at 4°C) at 3 000 rpm (1 200 g) for 30 min at 4°C. The plasma was then pipetted off into polystyrene tubes, and tested within 2 h.

Serum. Serum was prepared by incubating whole blood in glass tubes at 37°C for 1 h, centrifuging at 4°C for 15 min at 3 000 rpm (1 200 g). The freshly prepared serum was pipetted off and tested with ethanol in the same manner as citrated plasma.

Subjects

Patients admitted to Department IX, Internal Medicine, Ullevål Hospital, were examined on the first day of their stay irrespective of underlying disease.

Controls comprised 775 apparently healthy members of the medical staff, students and workers from a nearby plant. Their age ranged from 18 to 80 years.

Clinical diagnoses were assigned by the doctors in charge of the patients, without knowledge that this study was being performed. The diagnoses used in this department were in accordance with those of WHO (38). In patients suffering from more than one disease, only the main (first) diagnosis at discharge was registered. Registering the patients was done without knowledge of the results of the ethanol test.

Fatality. The patients were registered as dead or alive on the day of discharge.

The Ethanol Test

Platelet-poor citrated plasma, 0.5 ml, was transferred to [illegible] glass tube 70 [illegible] 10 mm. This sample was equilibrated for 3 min in [illegible] waterbath of 20°C. 0.15 ml of the ethanol solution was then added with a blow-out pipette. The tube was immediately shaken thoroughly to ensure adequate and rapid mixing. It was then left undisturbed. After 10 min the tube was tilted once slowly to the horizontal position and thus inspected for the presence of [illegible] gel.

The results were recorded as follows: Plasmas containing no grossly visible particulate matter and plasmas showing discrete granules or floccules only [illegible]ere considered as negative (Fig. 1 *a* and *b*). All plasmas showing a gel were considered as positive (Fig. 1 *c* and *d*).

Performance and evaluation of the ethanol tests was always done by the author who was unaware of the clinical condition of the patients.

Influence [illegible] f anticoagulant solution. Citrate is regularly used as anticoagulant in our department. Studies on 15 patients with [illegible] positive ethanol test revealed no difference whether citrate alone, hirudin/citrate or EDTA was used as anticoagulant. Using heparin/citrate th[illegible] gels were more coarse, but the results were essentially identical. For practical reasons the majority of the tests were performed on citrated plasma.

Table I *Number of patients in the various age groups*

Mean [illegible]ge of groups (positiv[illegible] and negative) calculated from coded mid-values (39)

Age group	Ethanol test Positive	Negative	Total
10–19	0	14	14
20–29	6	46	52
30–39	2	36	38
40–49	10	73	83
50–59	16	152	168
60–69	34	221	255
70–79	30	201	231
80–89	8	91	99
90–99	0	6	6
100–109	2	0	2
Total	108	840	948
Mean age (y.)	63.89	63.78	

Table II. *Diagnosis at discharge*

For calculation of χ^2 diseases of the blood, arteries and veins, including pulmonary embolism, have been combined, as have TB, other infectious diseases including sepsis, diseases of the genito-urinary tract, and miscellaneous. The expected numbers have been calculated from the proportion of positives $p = 108/948 = 0.1139$

WHO classification no.	Disease	Total no. of pats. tested	Ethanol test Positive Obs. ( )	Positive Exp. ( )	Negative Obs. (n)	Negative Exp. ( )
104–204	Malignant neoplasms	75	16	8.54	59	66.46
240–245	Allergy	42	2	4.78	40	37.22
250–260	Endocrine diseases	27	4	3.08	23	23.92
300–369 (330 334)	Mental and neurological diseases	68	0	7.75	68	60.25
330–334	Cerebro-vascular diseases	97	8	11.05	89	85.95
400–451 (–420.1 1/2)	Heart diseases	119	5	13.56	114	105.44
420.1–1/2	Acute myocardial infarction	112	11	12.75	101	99.25
474–527 (–490 –493)	Respiratory diseases	63	7	7.18	56	55.82
490–493	Pneumonia	76	18	8.66	58	67.34
539–587	Gastro-intestinal diseases	71	5	8.09	66	62.91
E870–E979	Intoxications	32	2	3.65	30	28.35
290–299	Diseases of the blood	13	4		9	
453–456	Diseases of arteries	4	1		3	
463–466 (–465)	Diseases of veins	5	3	3.42	2	26.58
465	Pulmonary embolism	8	2		6	
00–054	TB, other infectious diseases, sepsis	16	3		13	
590–637	Diseases of genito-urinary tract, uremia	18	4	15.49	14	120.51
	Miscellaneous	102	13		89	
	Total	948	108		840	

$\chi^2 = 54.87$ DF = 12; $p < 0.0005$.

RESULTS

A total of 1 807 patients were admitted to the department during the study of whom 948 (52.5%) were examined with the ethanol test, 108 (11.4%) demonstrating gelation of plasma, 840 (88.6%) being negative. No significant difference in mean age of the patients in the positive and the negative group was found (Table I).

The patients examined have been arranged according to disease categories (Table II). Assuming no diference in disease pattern of the positive and the negative group, the expected numbers of patients within these disease categories have been calculated (see note to Table II). A highly significant difference in disease pattern of the two groups was found, however. Main contributors to this difference are the relatively large number of malignant neoplasms, pneumonia and diseases of blood and blood-vessels, including pulmonary embolism, in the positive group. The absence of mental/neurological diseases and the relatively small number of heart diseases (Table II) in the positive group also add to this difference.

Deaths observed and expected among patients having a positive ethanol test have been recorded according to the various disease categories (Table III). The expected number of deaths in the positive group were computed for each disease category separately using case fatality of the corresponding disease category of the negative group (see note to Table III). The number of deaths in the positive group was significantly higher than in the negative.

Consistency of the ethanol test

A total of 108 patients, when first examined, demonstrated gelation of plasma. A new blood specimen, obtained within 24 h, was collected in

Table III. *Deaths observed and expected among patients having a positive ethanol test*

The expected number of deaths has been calculated separately for each disease category using case fatality among patients having a negative ethanol test

Diagnosis at discharge	Ethanol test positive: Patients ( )	Deaths: Obs.	Deaths: Exp.	Ethanol test negative: Patients (n)	Deaths: Obs.
Malignant neoplasms	16	8	4 07	59	15
Allergy	2	0	0	40	0
Endocrine diseases	4	0	0	23	0
Mental and neurological diseases	0	0	0	68	4
Cerebro-vascular diseases	8	2	1 17	89	13
Heart diseases	5	1	0.22	114	5
Acute myocardial inf	11	4	2.29	101	21
Respiratory diseases	7	1	0.13	56	1
Pneumonia	18	1	1.86	58	6
Gastro-intestinal diseases	5	2	0 15	66	2
Intoxications	2	0	0	30	0
Diseases of the blood	4	0	0	9	0
Diseases of arteries	1	1	0	3	0
Diseases of veins	3	0	0	2	0
Pulmonary embolism	2	1	0 33	6	1
TB, other infectious diseases, sepsis	3	2	0.23	13	1
Diseases of genito-urinary tract, uremia	4	0	0.57	14	2
Miscellaneous	13	0	0	89	0
Total	108	23	11.02	840	71

$\chi^2 = (23 - 11.02)^2/11.02 = 13.02$, DF = 1 $p < 0.0005$.

86 of these patients, 54 still demonstrating a positive test, 32 being negative. No significant difference in mean age or sex distribution of these two groups was found. Their diagnosis at discharge, however revealed a significant difference in disease pattern (Table IV). Main contributors to this difference were the relatively large number of patients with neoplasms and with pneumonia in the persistently positive group.

The ethanol test on serum

Serum was examined with the ethanol test in all patients demonstrating gelation of plasma, but was always found to be negative

Table IV *Consistency of ethanol test outcome by diagnostic groups*

The expected numbers have been calculated from the proportion of positives $p = 54/86 = 0.6279$

Diagnosis at discharge	Group total	Second test: Positive: Obs.	Positive: Exp	Negative: Obs.	Negative: Exp.
Malignant neoplasms	14	13	8.79	1	5 21
Pneumonia	16	12	10 04	4	5 96
Diseases of blood, arteries and veins, including pulmonary embolism	10	7	6 28	3	3 72
Others	46	22	28 88	24	17 12
Total	86	54		32	

$\chi^2 = 11.06$ DF = 3 $0.025 < p < 0.01$

The ethanol test in healthy controls

Plasma and sera from 275 healthy controls were tested, but were always negative.

DISCUSSION

In unselected patients admitted to our Department of Internal Medicine 11.4% of the patients demonstrated gelation of their plasma when examined with the ethanol test within 24 h after admission. By re-examining the positives within 24 h this incidence was lowered, as only 7.4% of all patients tested persistently showed gelation of plasma. In healthy controls, however, the test was uniformly negative. In no instance did the serum from patients give a positive test. This supports our biochemical evidence that ethanol gels mainly are composed of fibrinogen and fibrin (17). These findings substantiate the concept that gelation of plasma by ethanol indicates fibrinemia.

The lowered incidence of positives obtained when re-examining patients initially having a positive test suggests that fibrinemia may be transient in some patients. The reason why the test becomes negative within a few hours remains unsolved. Imperfect mixing of blood and anticoagulant might allow some fibrin to form. Prolonged stasis or poor blood flow may probably induce a positive test in some patients, although we could not demonstrate this in healthy controls. Blood-sampling through intravenous catethers may give a positive test. This is in accordance with thrombi formation in such catheters (14). Accordingly disposable needles should be used. Using such sampling technique, we feel that even a single positive ethanol test reflects hypercoagulability.

A preponderance of malignant neoplasms, pneumonia and diseases of blood and blood-vessels (Table II) was found among the patients having a positive test.

Coagulation studies in patients with malignant neoplasms have revealed multitude of changes, especially fluctuating levels of several coagulation factors, including fibrinogen (5, 6, 30). Increased amounts of cryofibrinogen have been demonstrated in these patients (16, 23, 31), as has the presence of split products (5). Several case reports have demonstrated consumption of coagulation factors, including fibrinogen in these patients (6, 10, 1–, 15, 22, 27). Presumably fibrinemia should occur in some of these patients. This is in agreement with our frequent finding of a positive ethanol test among cancer patients.

The high frequency of positive tests among patients with pneumonia may seem astonishing. It has been shown, however, that pneumonia is associated with intra-alveolar fibrin deposits (29) and extensive small vessel thrombosis (34).

The significantly increased number of deaths among patients having a positive ethanol test cannot be explained as due solely to the preponderance of lethal diseases (e.g. malignant neoplasms) in the positive group. In calculating the expected number of deaths in the positive group, an expected number of deaths within each disease category has been computed, using the case fatality of the corresponding negative group (Table III). Detailed analyses of the sex and age distribution of the patients within the different categories have not been made, however. Possibly the patients having a positive ethanol test have generally been more severely affected by their disease than those having a negative test. Intravascular coagulation is more prone to occur in severely ill patients (20, 21). Thus low grade intravascular coagulation, detectable by the ethanol test, may contribute to a fatal outcome.

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LIPOLYSIS AND GLUCOSE TOLERANCE IN OBESE SUBJECTS DURING PROLONGED STARVATION

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Abstract. Parameters reflecting the rate of synthesis and breakdown of triglycerides have been studied in subcutaneous adipose tissue removed under local anesthesia from eight subjects submitted to prolonged fasting in the treatment of obesity. The fasting period varied from 12 to 30 days and the mean daily loss of weight was approximately 0.5 kg. Sections of subcutaneous adipose tissue were incubated in albumin containing glucose-free Krebs-Henseleit bicarbonate buffer and in plasma obtained from the donor at the time of biopsy. Adipose tissue removed after fasting liberated glycerol into both types of medium twice as rapidly as tissue obtained before the fasting period. From the data on the uptake of radioactive palmitate and the re-esterification of FFA in tissue incubated in plasma, it can be assumed that the synthesis of triglycerides was rather well preserved during prolonged fasting. In spite of the increased lipolysis in subcutaneous adipose tissue in vitro, the plasma levels of FFA and glycerol at the time of the biopsies were almost the same before and during fasting. In a separate series of investigations, in which five obese subjects were submitted to one week's fasting, it was shown that the maximal glycerol production in subcutaneous adipose tissue exposed to isopropylnoradrenaline, dibutyrylcAMP and theophylline was the same before the start and at the end of this period. These findings indicate that the lipase activity and the response to beta-adrenergic agonists of adipose tissue were unchanged by one week's fasting. In contrast, noradrenaline had a definite antilipolytic effect at the end of the fasting period. Addition of the α-adrenergic blocking agent phentolamine to noradrenaline-containing medium resulted in a glycerol release similar to those evoked by dibutyrylcAMP and the other lipolytic agents used. Therefore, it may be assumed that the α-effect of noradrenaline, which inhibits lipolysis, is more pronounced in adipose tissue removed after prolonged fasting. For three subjects who initially had low k-values ($< 0.90\%$/min), as well as for three additional subjects with normal k-values before the fasting, the intravenous glucose tolerance increased with fasting. This improvement of the glucose tolerance could be explained by an increased peripheral utilization of glucose resulting from either higher sensitivity of peripheral tissue to insulin or from increased availability of insulin for tissues more sensitive to insulin than adipose tissue. The fall in plasma FFA and glycerol after glucose loading was less pronounced following fasting, an observation consistent with previous findings of decreased insulin secretion in the fasting state, although other mechanisms might coexist.

A major feature of the metabolic response to starvation in man is an accelerated lipid mobilization from fat depots as reflected in the increased level of FFA in plasma (12, 33). From studies on the assimilation of radioactive palmitate by isolated human subcutaneous adipose tissue, the accelerated mobilization of FFA was attributed to a diminished rate of esterification of FFA (23). This method, however, may give erroneous information about the rate of triglyceride synthesis both quantitatively and qualitatively (44). Therefore, it seemed appropriate to determine the production of glycerol as index of lipolysis and the re-esterification of FFA using the non-isotopic balance method of Vaughan (42) in subcutaneous adipose tissue removed from obese subjects before and during fasting.

In human as well as in rat adipose tissue, the breakdown of triglycerides is regulated by cyclic 3',5'-adenosine monophosphate (cyclic AMP). The concentration of this nucleotide is increased by noradrenaline and by substances which inhibit cyclic AMP phosphodiesterase activity in human adipose tissue (10, 16, 24, 45, 49). In previous studies (17), it was shown that the presence of glucose in vitro favoured the noradrenaline- and dibutyrylcAMP-induced lipolysis by separate mechanisms. Therefore, the second purpose of this study was to find out whether or not changes in the lipolytic response to noradrenaline in adi-

Table I. *Body height, weight, ideal weight calculated according to v. Döbeln and body composition calculated from total body potassium estimations of obese subjects submitted to variable length of fasting in the first study*

Pat. no	Sex	Age (y)	Height (cm)	Weight (kg)	Ideal weight (kg)	Body weight (% of the ideal weight)	Lean body mass (kg)	Fat mass (kg)	Fat mass (%)
1	♀	44	162	131.5	65.7	198.2	55.2	76.3	58
2	♀	32	164	117.5	73.0	172.0	54.3	63.2	54
3	♀	43	158	107.4	59.9	196.0	37.6	69.8	65
4	♀	38	174	164.2	80.4	200.4	77.1	87.1	53
5	♀	50	156	102.0	55.4	182.3	35.9	66.1	65
6	♂	51	186	107.0	83.5	126.5	60.8	46.2	43
7	♂	60	184	1 1.0	81.0	146.8	64.3	54.7	45
8	♂	43	182	99.5	82.4	135.4	47.7	51.8	52

pose tissue occurred also during prolonged fasting.

Finally, the glucose tolerance and the fall in plasma FFA and glycerol after intravenous glucose loading were determined before and during starvation when a significant reduction in the body weight of the obese subjects had been achieved. The aim of this part of the investigation was to elucidate the possible role of elevated plasma FFA as a major factor in the decrease of glucose tolerance in obesity, an observation first made in the early 1920's (34).

MATERIAL AND METHODS

First study

This included eight subjects (5 female and 3 male) referred to a medical clinic because of obesity without clinical evidence of endocrine disorder. One subject had elevated plasma levels of triglycerides, cholesterol and phospholipids corresponding to Type IV of hyperlipoproteinemia, according to the classification of Fredrickson et al. (20). Since the changes in FFA metabolism and glucose tolerance were indistinguishable from those observed in other subjects, he was included in the series. The ages, body weights, heights, ideal weights, and body compositions are presented in Table I. The patients were weighed on standard scales, accurate to within 0.1 kg. The maximal normal body weight was estimated from the height and wrist widths as suggested by v. Döbeln (15). Body composition was calculated from the total body potassium determined in a whole body counter (11, 19) and calibrated for weight as described previously (27, 28). All patients had been on unrestricted diets before entering the study. None had been subjected to recent attempts at weight reduction in the months immediately prior to the start of the study. During the prefasting period they were maintained on the general hospital diet containing about 2 200 kcalories per day.

On two occasions, before and after variable length (12–30 days) of fasting, biopsy specimens of subcutaneous adipose tissue were taken and intravenous glucose tolerance tests were performed. During this period the patients received calorie-free liquids, multivitamin capsules, and, if necessary, potassium chloride, sodium bicarbonate and antacids. No serious complications were observed. Four women developed hypopotassaemia (2.–3.3 mEq/l), easily corrected by oral supplements of potassium chloride. At the time of the second glucose tolerance test, the serum potassium levels were normal in all but one (no. 1), who also developed T-flattening of the ECG at one time. Otherwise, there were no ECG changes noted. Two women received sodium bicarbonate to correct slightly decreased serum bicarbonate levels. Daily determinations of the urinary excretion of acetoacetate by the Ketostix method (Ames Company) indicated that the calorie deprivation was total in all subjects during the whole period.

Biopsy procedure and glucose tolerance test. With the patient in recumbent position the biopsy area, the upper lateral part of the thigh, was anaesthetized with approximately 10 ml of 1% prilocain chloride (Citanest®, Astra). A polyethylene catheter was inserted into a brachial vein. Ten minutes later biopsy samples of adipose tissue, approximately 5 g each, were removed with almost no discomfort through a 2 cm long incision. The tissue sections were divided into sections of about 50 mg each and placed in 10 ml of Krebs-Henseleit bicarbonate buffer with human serum albumin (Kabi) and kept at 37°C for one hour. Fifteen minutes after wound closure, venous blood samples were taken into heparinized tubes for determination of blood glucose, plasma FFA, and glycerol. In addition, two 20 ml samples of blood were taken into syringes containing 1 ml of Na_2 EDTA (1.34 g/100 ml saline) to obtain plasma for incubation of adipose tissue. 50% aqueous glucose was then injected rapidly in a contralateral brachial vein in a dose of 0.25 g/kg b.wt. Venous blood samples were removed at 5-minute intervals for glucose determinations, and at 10-minute intervals for plasma FFA and glycerol determinations.

Incubation procedure. After 1 h of preincubation, the dissected fat portions of about 50 mg, eight, were distributed among three albumin and three plasma media. The albumin medium consisted of 6 ml of Krebs-

Table II. *Changes in body weight, plasma FFA, plasma glycerol, glucose tolerance and glucose disposal rate in eight subjects during prolonged fasting*

Pat. no.	Fasting period (d.)	Loss of weight (kg)	Plasma FFA (mM/l) initial	Plasma FFA (mM/l) fasting	Plasma glycerol (mM/l) initial	Plasma glycerol (mM/l) fasting	Glucose tolerance k-value (%/min) initial	Glucose tolerance k-value (%/min) fasting	Glucose disposal rate (g/50 min) initial	Glucose disposal rate (g/50 min) fasting
1	27	15.1	0.86	1.32	0.157	0.237	1.20	1.39	26.5	24.0
2	19	10.9	1.34	1.00	0.164	0.070	1.61	1.76	20.1	21.9
3	23	12.0	1.46	1.02	0.296	0.254	0.82	1.02	14.0	16.5
4	24	13.4	1.11	1.29	0.167	0.181	0.37	1.31	11.1	23.3
5	30	9.4	1.45	0.99	0.270	0.166	1.12	1.22	15.1	14.2
6	12	6.6	1.21	1.20	0.205	0.14[illegible]	1.17	0.88	18.4	14.1
7	20	7.9	0.48	1.05	0.220	0.209	1.24	1.73	21.0	25.6
8	23	9.3	0.91	0.84	0.191	0.161	1.73	1.55	23.5	16.5

Henseleit bicarbonate buffer (pH 7.4) with 4 g/100 ml of human serum albumin added. In the plasma medium Na_2 EDTA was used in an amount known to interfere neither with the determinations of FFA and glycerol nor with the metabolic parameters measured (47). ^{14}C palmitate (Amersham, England) was purified according to Borgström (6). The neutralized palmitate solution was warmed until optically cleared and then diluted with albumin-containing Krebs-Henseleit bicarbonate buffer before being dissolved into either albumin or plasma medium.

After 2 h of incubation aliquots of the medium were removed for determination of FFA (13, 41) and glycerol (29, 46). Tissue FFA, lipid weight, neutral lipid and FFA radioactivities were determined in homogenized tissue as described in detail previously (47). Re-esterification of FFA was calculated as three times glycerol productions minus total FFA production, i.e. net changes in the concentrations of FFA in tissue plus medium.

Glucose tolerance test. Blood glucose was determined according to Marks (32). The slope of the semilogarithmic decline of blood glucose with time (k-value in 10^{-2} min^{-1}) was determined according to Ikkos and Luft (25). The amount of glucose disposed from the "glucose space" within 50 min after injection was calculated by a formula similar to that described by Streeten et al. (39). Glucose disposal rate (g/50 min)

$$= \frac{\text{glucose load}}{BG_0' - BG_0} \cdot BG_0 - BG_{50}$$

where BG_0' = blood glucose intercept at zero-time, BG_0 = blood glucose concentration at zero-time, BG_{50} = blood glucose concentration at 50 min. No correction for glucosuria was made.

Second study

Five obese but otherwise healthy subjects were included (2 male and 3 female). Their weight ranged from 118 to 245% of the predicted values. Immediately before and after 7 days following fasting, subcutaneous adipose tissue was removed as described above. In separate experiments it was ascertained that rapid washing of tissue was essential to prevent the inhibitory effect of the local anaesthetic agent on the stimulated lipolysis in human adipose tissue.

Incubation procedure. Basal incubation medium consisted of 3 ml of Krebs-Henseleit bicarbonate buffer with 3% of bovine serum albumin (Fraction V, Armour, England, lot no. MG 2770) and 1 mg/ml of glucose. Incubations were run in duplicate for 2 h. Glycerol determinations were made according to Wieland (46) as modified by Larsen (29). Glycerol release was expressed per unit wet weight. The following agents were added in concentration known to produce maximal glycerol release: noradrenaline bitartrate (Astra), isopropyl-noradrenaline sulphate (Sterling Winthrop Research Institute), N⁶2'O-dibutyrylcAMP (Boehringer/Ingelheim) and theophylline. Phentolamine HCl, as the pure substance (Ciba), was added at two different concentrations to noradrenaline-containing medium. These concentrations were chosen as it has been observed both in published (16, 50) and unpublished studies that they reduced maximal lipolysis. Statistical analysis was performed according to Snedecor (38).

RESULTS

In the group of eight obese individuals submitted to varying lengths of fasting the loss of body weight ranged from 6.6 to 15.1 kg (Table II) with a mean daily loss of about 0.5 kg. In all but one subject the prefasting levels of plasma FFA and glycerol were higher than usually observed in non-obese subjects. As can be seen in Table II, the fasting condition had a variable but small influence upon the plasma concentration of FFA. Significant increases were observed only in two subjects. A certain parallelism between the changes in plasma FFA and plasma glycerol appeared.

In Table III are shown the data reflecting the rates of production and breakdown of trigly-

Table III. *Effect of prolonged fasting on parameters reflecting the production and the breakdown of triglycerides of human subcutaneous adipose tissue in vitro*

Mean of eight experiments ± standard error. Results are expressed as μmoles/g lipid weight/2 h. Albumin medium: 6.0 ml Krebs-Henseleit bicarbonate buffer with 4% of human serum albumin. Plasma medium: 6.0 ml of plasma removed at time of biopsy and Na_2-EDTA used as anticoagulant. Palmitic-acid-^{14}C was added in an amount providing approximately 5×10^6 cpm/ml. Re-esterification: Glycerol release × 3 – net production of FFA in tissue plus medium. FFA uptake calculated from the radioactivity recovered as total lipids divided by the mean specific activity of medium FFA estimated according to Dole.

Incubation medium	Tissue FFA	Medium FFA	Medium Glycerol	Re-esterification	FFA uptake
Albumin					
Before fasting	−0.29±0.30	+0.79±0.41	+0.32±0.07	0.46±0.40	0.31±0.05
After fasting	−0.70±0.40*	+2.76±0.64	+0.66±0.14	0.04±0.46	0.22±0.05
Plasma					
Before fasting	−0.80±0.37	+0.17±0.37	+0.52±0.07	2.17±0.54	0.62±0.08
After fasting	−1.90±0.52	+2.36±0.34	+0.85±0.20*	2.08±0.59	0.58±0.09

Significance between findings before and after fasting: * $p<0.05$ $p<0.005$ $p<0.001$

cerides in subcutaneous adipose tissue removed before and during fasting and incubated in either glucose-free albumin medium or in plasma obtained from the donor at the time of biopsy. The net release of FFA and glycerol into both types of medium were significantly more rapid from tissue taken during fasting. From the total uptake of radioactive palmitate and the calculated re-esterification of FFA, it may be concluded that the amount of FFA used for triglyceride synthesis in tissue incubated in plasma was unchanged by prolonged fasting, whereas the capacity to utilize FFA tended to be low in adipose tissue exposed to albumin milieu. In general the uptake of FFA was higher and the total production of FFA lower in adipose tissue incubated in the plasma medium compared with the albumin medium. This could be due to the higher concentration of FFA and/or the presence of glucose and probably insulin in this type of medium.

Fig. 2 shows that the lipolysis produced by

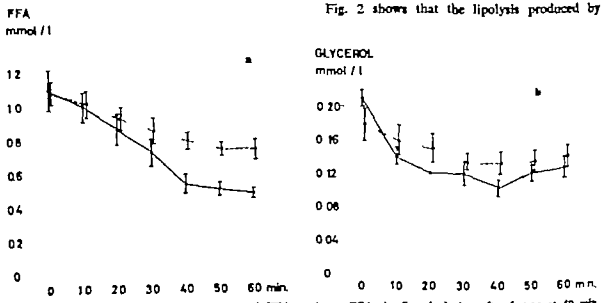


Fig. 1. Changes in the plasma concentration of FFA (a) and glycerol (b) during intravenous glucose tolerance test in eight obese subjects before (—) and at the end of fasting (12–30 days) (O--O). Mean ± standard error plasma FFA significantly higher after fasting at 40 min ($p<0.05$), 50 min ($p<0.05$), plasma glycerol significantly higher after fasting ($p<0.05$ at 10 min) (p 0.02 at 20 min) ($p<0.05$ at 40 min).

each of the agents isopropylnoradrenaline, theophylline and dibutyrylcAMP was somewhat higher in adipose tissue removed after one week's fasting than before this period, the responsiveness being insignificant when calculated from the paired differences. In four out of the five prefasting experiments noradrenaline stimulated lipolysis (Fig. 3). Rather unexpectedly noradrenaline inhibited the glycerol release from tissue of all five subjects submitted to fasting. This difference in lipolytic response to noradrenaline between tissue removed before and after fasting was highly significant ($p < 0.001$). The addition of phentolamine to medium containing noradrenaline increased the glycerol production in adipose tissue whether removed before or after fasting. In the fasting state the maximal glycerol release induced by noradrenaline plus the most effective of the two concentrations of phentolamine was 2.91 ± 0.39/μmoles/g/2 h (mean $\pm$ S.E.) which is slightly less than the mean figure obtained with isopropylnoradrenaline and the other lipolytic agents as shown in Fig. 3

In six out of eight subjects the glucose tolerance unexpectedly increased during fasting. The increases in glucose tolerance as judged from the k value and the disposal rate of glucose were small, but in one very obese subject the *k* value rose from 0.37 to 1.31% min In most sub-

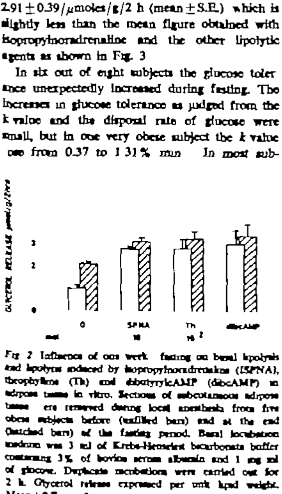


Fig. 2 Influence of one week fasting on basal lipolysis and lipolysis induced by isopropylnoradrenaline (ISPNA), theophylline (Th) and dibutyrylcAMP (dibcAMP) in adipose tissue in vitro. Sections of subcutaneous adipose tissue were removed during local anaesthesia from five obese subjects before (unfilled bars) and at the end (hatched bars) of the fasting period. Basal incubation medium was 3 ml of Krebs-Henseleit bicarbonate buffer containing 3% of bovine serum albumin and 1 mg ml of glucose. Duplicate incubations were carried out for 2 h. Glycerol release expressed per unit lipid weight. Mean $\pm$ S.E. – 5

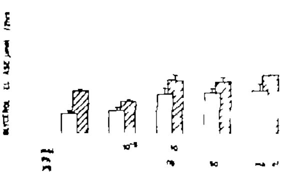


Fig. 3 Influence of one week's fasting on lipolysis induced in subcutaneous adipose tissue by noradrenaline (NA) alone or in combination with phentolamine (Ph). The last pair of bars denote the maximal lipolysis obtained by noradrenaline and any of the two concentrations of phentolamine Experimental conditions given in Fig. 2.

jects the changes in the rate of glucose disposal run parallelly with the changes in the *k* value. A decreased glucose tolerance was observed in only two subjects (nos. 6 and 8). In both of them the loss of body weight, 6.6 and 9.3 kg respectively was lower than the mean figure of 10.9 kg in the other subjects. Several months after this study and at a time when the body weight lost during fasting had been regained, a third glucose tolerance test was performed on the six subjects who had exhibited improved glucose tolerance during fasting. In five subjects the glucose tolerance had diminished.

The fall in plasma FFA after glucose loading was significantly less after fasting ($p < 0.05$ at 40 and 50 min after glucose administration, Fig. 1). Correspondingly the concentration of plasma glycerol decreased less abruptly after glucose administration at the end of the fasting period ($p < 0.05$ at 10 min, < 0.02 at 20 min and < 0.05 at 40 min)

DISCUSSION

Adipose tissue metabolism

Present data show that during prolonged fasting there is as an average twofold increase of glycerol production in subcutaneous adipose tissue incubated either in albumin or plasma medium. In contrast, the re-esterification of FFA appears to be well preserved during prolonged fasting. Further support for unchanged assimilation of FFA in the fasting state is given by the findings

of an unchanged rate of uptake of radioactive palmitate into tissue incubated in plasma. These findings differ significantly from those previously reported in subcutaneous adipose tissue removed from diabetics in demand of insulin, in which decreased formation as well as accelerated break down of triglycerides were observed (48) On the basis of these two studies it seems likely that only small amounts of insulin are required for an adequate uptake and esterification of FFA in human adipose tissue. The more rapid uptake of FFA in adipose tissue incubated in plasma in comparison with albumin medium might also reflect the influence of insulin on these metabolic processes. No proof of this, however is actually given, since the two types of incubation medium differ also in their content of glucose, occurring only in the plasma medium.

The accelerated release of glycerol from adipose tissue obtained after prolonged fasting was not surprising, since parallel increases in the plasma levels of FFA and glycerol have been reported during the initial phase of fasting in non-obese subjects. In the present work the plasma concentrations of FFA and glycerol were, for most of the eight subjects, nearly the same before and after the period of fasting. This indicates that the increased lipolysis is balanced by an increased efflux of FFA and glycerol.

A number of hormones such as the catecholamines, glucagon and growth hormone (8) may be responsible for the increase in lipid mobilization from fat stores during fasting. Another possible explanation of the increased lipolysis during fasting in man would result from a reduced supply of insulin secondary to a consistently low blood sugar and, thus, increased production of cyclic AMP in adipose tissue. At present it is not known which of these hormonal influences is most important in determining the rate of lipolysis in human adipose tissue during fasting. Furthermore, it is not known whether or not identical mechanisms may exist in the early and the later phases of prolonged fasting. In this study the maximal lipolysis induced by isopropylnoradrenaline, theophylline and dibutyrylcAMP was essentially unchanged by fasting. These findings indicate that no appreciable changes in the lipase activity occur either in the subcutaneous adipose tissue of obese human subjects or in the epididymal fat pad of the rat submitted to fasting.

A certain difference, however seems to exist between these tissues in their lipolytic response to noradrenaline. Whereas in rat adipose tissue the lipolytic response to noradrenaline was augmented by fasting, the glycerol release in human subcutaneous adipose tissue was inhibited by this agent. This discrepancy would be explained by assuming that the α-adrenergic effect of noradrenaline is much weaker in rat than in human adipose tissue. In man the existence of α- and β-adrenergic receptors is well established, and this dual mechanism of the catecholamines might well be of great significance for the transport of energy in man. The significance of this antilipolytic effect of noradrenaline could thus be the assurance that the adipose tissue will *not* be deprived of its triglycerides during fasting. At present it is not known whether noradrenaline acts in an antilipolytic fashion also in adipose tissue of non-obese subjects as well as in adipose tissue found in other parts of the body

Glucose tolerance

In obese subjects the decreased glucose tolerance and resistance to endogenous insulin (31) have been attributed to decreased sensitivity of adipose tissue to insulin, diminished uptake of glucose by muscle (35) because of for example, elevated plasma FFA (36), and to an insufficient supply of insulin for muscle because of the increased fat mass (7). Whereas reduction in the body weight is known to improve glucose tolerance in obese subjects (4 18, 26, 30, 37), starvation periods of up to 12 days duration have resulted in diminished carbohydrate tolerance in non-diabetic, obese individuals (21 40).

In the present study the glucose tolerance decreased only in two out of eight subjects. Although no appreciable alterations in the plasma levels of FFA and glycerol were seen, there is little doubt that the influx of FFA was significantly greater at the time of the second biopsy and glucose tolerance. Assuming that the in vitro data from the subcutaneous adipose tissue were representative of adipose tissue at other localizations, it can be concluded that the total outflow of FFA was considerably higher after *fasting*, since the increase in FFA liberation per unit weight of adipose tissue was relatively more pronounced than the decrease of the fat mass. The discrepancy between our and previous data could,

therefore, be explained by the longer period of fasting we have used and by the concomitant loss of body fat. Furthermore, our care to normalize the serum levels of potassium and bicarbonate might have been important (1). Total body potassium determinations, however revealed a potassium loss in all patients. The mean loss was 21 7 g (range 9 0–49 4 g). In the present study no measurement of body fat loss was made. From data by Ashley and Whyte (2) and Gilder et al. (22) using metabolic balance and indirect calorimetry it can be roughly calculated that the fat loss during starvation in our patients was about 4.5–10 kg. Thus a short period of fasting would result in accelerated lipid mobilization, decreased response of insulin to glucose and decreased glucose tolerance. A longer period of fasting, on the other hand, with significant reduction of body weight, would result in smaller fat cells and loss of fat mass, which counteracts the inhibitory effect of elevated plasma FFA on peripheral utilization of glucose. In addition, a certain loss of fat mass leads to a reduction in the total outflow of FFA and might make more of the insulin secreted by the pancreas available for other tissues requiring insulin.

It remains to be decided which of these factors is most important for the improved glucose tolerance observed in this study Our data, however suggest that the decreased glucose tolerance and diminished insulin sensitivity in obesity cannot be explained by an increased mobilization of FFA from fat depots.

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HEMODYNAMIC EFFECTS OF TOLAZOLINE

Studies in Normal Subjects and Patients with Mitral Valvular Disease, Congenital Heart Disease and Primary Pulmonary Hypertension

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Abstract. Right and left heart catheterization has been carried out before and after injection of tolazoline in 5 normal subjects and in 32 patients with mean pulmonary arterial pressure of 20 mmHg or more. A solution of tolazoline, 1 mg/kg b.wt., was injected during one minute in the pulmonary artery. In the patients an increase in oxygen consumption and reduction in the arteriovenous oxygen difference was observed. Both changes were significant. Consequently cardiac index increased significantly. There was significant rise of the heart rate. The mean pressure of the pulmonary artery and in the pulmonary wedged position revealed almost no change. The systolic systemic pressure initially fell slightly but after ten minutes values at original level were recorded. Systemic arteriolar resistance was significantly reduced. In the normal subjects principally the same changes were observed. It is concluded that tolazoline usually is ineffective as drug in reducing pulmonary arterial pressure in pulmonary hypertension.

Tolazoline (priscoline hydrochloride) is an adrenergic blocking agent (8, 9, 11). By an effect on the smooth vascular musculature it also produces local vasodilatation (4). In a series of investigations the injection of tolazoline has been found to give rise to a reduction of the pulmonary arterial pressure (1, 2, 3, 4, 5, 6, 10, 16, 17, 18), but in other studies almost no change (1, 12) and even a pressure increase (7, 12) has been reported. The effect on the pulmonary arteriolar resistance has been different in the investigations reported.

Further studies of the hemodynamic effects of tolazoline therefore seem to be of interest.

MATERIAL

The investigations were performed in 5 normal subjects and in 32 patients with mean pulmonary arterial pressure at rest of 20 mmHg or more. Twenty-two of the patients had mitral valvular disease, eleven of them also had affections of other valves. The remaining patients were six with congenital heart disease and four with primary pulmonary hypertension. In the normal group there were three men and two women, average age 17 years, in the patient group 15 men and 17 women with an average age of 46 years.

METHODS

Right heart catheterization was carried out with Cournand catheter. On the left side polyethylene catheter (32 cases) or thin teflon catheter (5 cases) was introduced into the femoral artery using the Seldinger technique or modification of it. In one patient with mitral stenosis third catheter was placed in the left atrium by transseptal technique, and in another patient with ventricular septal defect three catheters were placed in the right atrium, pulmonary artery and aorta, respectively. The pressures were recorded with Elema transducers. Cardiac output was calculated according to Fick's principle. The volume of expired air was measured with Tissot spirometer, and oxygen consumption was determined from gas analyses by the micromethod of Scholander. Blood oxygen saturation was measured with Unicam spectrophotometer.

A solution of tolazoline, 1 mg/kg b.wt. in 20 ml 0.9% sodium chloride, was injected during one minute in the catheter into the pulmonary artery. Pressures were recorded in the pulmonary artery, wedged position (in one case in the left atrium) and aorta (30 cases) or left ventricle (7 cases) before injection and ten minutes after the injection. In ten unselected patients with mitral valve disease the diastolic pressure gradient across the mitral ostium was measured before and about twenty minutes after the injection. In two patients the effect of tolazoline was examined both at rest and during exercise on an ergometer bicycle.

Table I. *Hemodynamic effects of tolazoline in patients*

	Before			3 min after			10 min after		
		Mean	S.D		Mean	S.D		Mean	S.D.
Heart rate (beats/min)	32	83	16	31	111	26	32	102	25
Pulmonary wedge pressure (mean, mmHg)	23	17	8	21	20	13	23	19	11
Pulmonary arterial pressure (mean, mmHg)	32	43	14	31	43	16	32	43	16
Systolic systemic pressure (mmHg)	32	130	23	31	121	21	32	129	19
Oxygen consumption (ml/min)	31	230	46				31	266	51
Arteriovenous oxygen difference (ml/l)	26	60.9	14.8				26	51.6	12.5
Cardiac index (l/min/m²)	26	2.3	0.6				26	3 1	0 8
Stroke volume (ml)	26	50	20				26	55	23
Pulmonary arteriolar resistance (dynes sec cm⁻⁵)	22	407	357				22	267	220
Systemic arteriolar resistance (dynes sec cm⁻⁵)	19	2 273	1 099				19	1 500	303
Diastolic pressure gradient across mitral valve (mmHg)	10	16	9				10	24	11
Pulmonary blood flow (in cases with left-to-right shunt) (l/min)	6	9.6	6.3				6	9.6	4.8
Arterial oxygen saturation (%)	31	91	3.8				31	90	4.3

RESULTS

The results are presented in Tables I and II. In the patients there was observed an increase in oxygen consumption and a reduction in arteriovenous oxygen difference. Both changes were highly significant on comparison with the corresponding values before injection. Consequently there was a significant increase in cardiac index, from an average of 2.3 l/min per m² BSA to 3 1 Both at three and ten minutes after injection, but especially great after three minutes, there was a significant increase in heart rate. Stroke volume showed an insignificant increase. The rise of cardiac index was therefore mainly caused by the increase in heart rate. The mean pressure in the pulmonary artery and in wedged position revealed almost no change. The systolic systemic pressure fell slightly but significantly three minutes after injection, but after ten minutes nearly starting values were recorded.

Both the systemic and pulmonary arteriolar resistances were significantly reduced, in particular the systemic resistance. Tolazoline injection did not change the arterial oxygen saturation. In the ten mitral stenosis patients there was a significant rise of the diastolic pressure gradient across the mitral valve, on an average from 16 to 24 mmHg. In this patient group there was also a significant increase of the wedge pressure both after three and ten minutes.

When the ten patients with congenital heart disease and primary pulmonary hypertension were treated statistically as one group, the hemodynamic data were largely similar to those of the entire material. Only one of the four patients with primary pulmonary hypertension had a definite fall of pulmonary arterial pressure after tolazoline injection.

In the six patients with left-to-right shunt no significant change of the pulmonary blood flow was found. In only one of these cases was a catheter placed in the right atrium, and in this patient there was a distinct rise of pulmonary blood flow with an increase of the left-to-right shunt.

In two patients (one with mitral stenosis and one with primary pulmonary hypertension) the effect of tolazoline was examined at rest and during exercise on an ergometer bicycle with a load of 200 kpm/min in the supine position. In both cases tolazoline gave a greater increase in heart rate during exercise. Pulmonary wedge pressure was measured only in the patient with mitral stenosis. There was an increase from 15 to 26 mmHg and from 27 to 32 mmHg at rest and on

Differences

3 min after–before injection					10 min after–before injection				
	Mean	S.E.	p	t		Mean	S.E.	p	t
31	28.2	3.25		8.69	32	20.1	2.75		7.32
21	3.3	2.03	—	1.64	23	1.9	1.70	—	1.12
31	0.4	1.88	—	0.22	32	0.6	1.82	—	0.34
31	9.4	2.99		3.14	32	0.8	2.46	—	0.31
					31	36.4	5.11		7.12
					26	9.3	2.48		3.73
					26	0.8	0.13		6.16
					26	4.8	3.54	—	1.37
					22	139.2	49.6		2.81
					19	773	196.7		3.93
					10	8.1	2.07		3.91
					6	0.03	1.66	—	0.12
					31	0.61	0.18	—	1.61

= 0.05 > p > 0.01, = 0.01 > p > 0.001 = p < 0.001

exercise, respectively. Pulmonary arterial pressure was almost unchanged at rest. Only in the patient with primary pulmonary hypertension was the mean pressure during exercise reduced, from 88 mmHg before injection to 60 mmHg after tolazoline had been given. In this patient the cardiac index decreased slightly whereas it rose distinctly in the other case. Pulmonary arteriolar resistance could only be calculated in the patient with mitral stenosis, who showed a definite reduction both at rest and during exercise after tolazoline.

In the five normal subjects small changes were recorded, but principally very similar to those in the patient group. Only heart rate and cardiac index increased significantly and the t values were lower than in the patients. Systolic systemic pressure did not change. Pulmonary arteriolar resistance (which could be calculated only in the mitral stenosis patients) increased slightly.

No serious complications were observed after the injections of tolazoline. The majority of the subjects examined had a sense of warmth and a visible flushing about half a minute after the start of the injection, soon followed by a chilly sensation. Some patients felt unpleasant when the heart rate increased, especially the patients with mitral valve disease in whom the wedge pressure also rose. One patient with mitral stenosis and bronchial asthma had a feeling of retrosternal pressure, which soon disappeared.

DISCUSSION

Earlier publications (1, 2, 3, 4, 5, 6, 10, 16, 17, 18) mostly report a reduction of the pulmonary arterial pressure following injection of tolazoline. Kelminson et al. (6) found that the combination of 100% of oxygen and tolazoline gave a greater reduction of the pulmonary arterial pressure than tolazoline alone. In this material of patients with pulmonary hypertension of various etiology almost no change was observed. Similar findings have been reported by others (1, 7, 1.). In newborn calves with artificial pulmonary hypertension a rapid fall in the mean pulmonary arterial pressure was found after tolazoline had been injected, but the value returned to control level within three minutes. Mackinnon et al. (7) observed in one patient a sharp rise of the pulmonary arterial pressure and a fall in cardiac output. In another case their examinations were

Table II. *Hemodynamic effects of tolazoline in normal subjects* ($n = 5$)

	Before		3 min after		10 min after		*Differences* 3 min after–before				10 min after–before			
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.E.	p	t	Mean	S.E.	p	t
Heart rate (beats/min)	84	11.5	108	14.0	101	16.3	24	4.3		5.64	17	3.7		4.54
Pulmonary wedge pressure (mean, mmHg)	5	1.5	4	1.3	3	2.1	0.2	0.4	—	0.53	1.4	0.5	—	2.75
Pulmonary arterial pressure (mean, mmHg)	8	1.1	7	1.5	9	4.1	0.6	0.4	—	1.50	1.2	1.4	—	0.84
Systolic systemic pressure (mmHg)	109	23	103	14	106	21	5.6	5.1	—	1.09	2.4	3.0	—	0.80
Oxygen consumption (ml/min)	227	44			255	62					27	13.3	—	2.04
Arteriovenous oxygen difference (ml/l)	34.6	3.8			29.5	3.4					5.1	2.4	—	2.09
Cardiac index (l/min/m²)	3.9	0.8			5.0	0.5					1.1	0.3		4.26
Stroke volume (ml)	82	21			87	23					4.2	8.1	—	0.52
Pulmonary arteriolar resistance (dynes sec cm⁻⁵)	41	8			52	16					11	8	—	1.37
Systemic arteriolar resistance (dynes sec cm⁻⁵)	1 038	273			757	190					281	106	—	2.65

$= 0.01 > p > 0.001$ $= 0.05 > p > 0.01$

not accomplished on account of a similar response.

Some of the patients in our material did not feel well during the first minutes following injection of tolazoline. This occurred especially in patients with a great increase in heart rate, pulmonary arterial pressure and diastolic pressure gradient across the mitral valve. One patient developed a retrosternal pressure which soon disappeared. Prolonged cardiac pain following intra-arterial injection of tolazoline has been previously reported (13).

In the present material no significant change in the pulmonary arterial wedge pressure was recorded. This has, however been found by others (4). In accordance with previous reports (3 5 6, 16) we observed a highly significant increase in cardiac index. No change of cardiac output has also been reported (7). In the present study there was a very slightly significant decrease of the pulmonary arteriolar resistance. Most investigators (3 4 6, 16 17) have found a reduction, but no change in the pulmonary arteriolar resistance has been reported (7) Grover et al. (4) found a decrease of the systemic arterial pressure, and in the present study there was also observed a significant pressure fall of short duration. Rudolph et al. (12) emphasized the largely parallel change in the systemic and the pulmonary arterial pressures. Corresponding with other publications, the present investigation revealed a highly significant fall of the systemic arterial resistance. As reported by Mackinnon et al. (7), this study also showed a highly significant rise in heart rate. No change of arterial oxygen saturation was found, corresponding with previous findings (16) whereas others (14) have found a decrease.

Dresdale et al. (3) described a patient who could not achieve more than 16 step-ups on the platform used in their pulmonary function studies because of precordial distress and weakness, but was able to do more than 70 step-ups without symptoms following the intravenous administration of 75 mg tolazoline. As far as can be found in the literature, no other studies of tolazoline during work have been performed. In the present study two patients were exercising on a bicycle ergometer before and after injection of tolazoline. In both cases the heart rate increase was greater when tolazoline and exercise were combined. Only in one patient with primary pulmonary hypertension was the mean pulmonary arterial pressure reduced during work after tolazoline administration. Pulmonary arteriolar resistance could be calculated in one patient, in whom a distinct reduction both at rest and during exercise following tolazoline injection was found.

In the normal series there was a slightly sig-

nificant increase in heart rate and cardiac index. The other changes were insignificant. In normal subjects Grover et al. (4) observed only a mild tachycardia but no definite alteration in pressures and cardiac output.

The most important effect of tolazoline seems to be vasodilatation (11). Cardiac output, however, is increased, and therefore the reduction in systolic systemic arterial pressure is moderate. The pulmonary arterial resistance is slightly reduced, and in most cases the increased cardiac output does not raise the pulmonary arterial pressure.

CONCLUSION

The first optimistic reports of reduction of the pulmonary arterial pressure in pulmonary hypertension by tolazoline have not been confirmed in this investigation. After injection of tolazoline the pulmonary arteriolar resistance has been shown to be moderately reduced, but cardiac output increased, and on an average no decrease in pulmonary arterial pressure was observed. It may be concluded that tolazoline has no significant effect in reducing pulmonary arterial pressure in pulmonary hypertension.

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LEFT TO-RIGHT SHUNT IN ATRIAL SEPTAL DEFECT AT REST AND DURING EXERCISE

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Abstract. The pulmonary and systemic flow has been calculated at rest and during exercise in 16 patients with atrial septal defects. In 14 of these patients there was normal pulmonary arterial pressure at rest. Remarkably many of the patients showed only slight increase in the systemic flow during exercise. The pulmonary flow increased during exercise in most of the patients. The absolute left-to-right shunt fell in 5 patients, was unchanged in 1 and moderately increased in 10 patients. No correlation was found between the magnitude of the shunt and the variation in the pulmonary blood flow during exercise. The pulmonary flow ratio decreased in all patients except 3 during exercise.

According to clinical experience it is remarkable that young patients with atrial septal defect (ASD) and even large left-to-right shunts are able to perform hard physical exercise and sport. An important question in this connection is: How is the absolute and the relative size of the shunt during exercise as compared to that at rest?

Several studies have shown that the physical working capacity is not correlated to the magnitude of the left-to-right shunt in patients with uncomplicated ASD (1 3 5). But only a few studies have been published on the behaviour of the shunt during physical work (2, 7 9). The results of these investigations have been conflicting.

The aim of the present study is to try to throw further light on this particular problem.

MATERIAL

Sixteen patients with ASD of the ostium secundum type (8 women and 8 men, aged 14 to 52 years) ere studied by right heart catheterization. All patients showed significant increase in oxygen saturation from the caval veins to the right atrium, and all patients had positive hydrogen test in the right atrium, negative in the superior caval vein.

METHODS

Right heart catheterization was carried out with hydrogen electrode Cournand catheter inserted into the basilic vein. A thin teflon catheter was introduced into the brachial or femoral artery by modified Seldinger technique. A polyethylene catheter was introduced by Seldinger technique through an antebrachial or femoral vein into the superior or inferior caval vein. The patients ere primarily examined in the supine position at rest. Pressures were measured in systemic arterial, pulmonary wedged and pulmonary arterial positions. Samples of blood ere taken from systemic and pulmonary artery and from inferior and superior caval vein after moving the catheter. Oxygen saturation in the blood samples was measured according to the principle of Gordy and Drabkin (4) by use of Unicam spectrophotometer. Expired air as collected for 3 min in Douglas bag. Gas analysis of the samples of expired air as done by the Scholander micro-method. The volume of expired air was determined with gasometer and converted to STPD.

The catheters were then replaced in their original positions. Exercise was carried out in the supine position on bicycle ergometer with loads varying from 150 to 200 kpm/min for 8 to 11 min. Pressures were recorded every minute in systemic and pulmonary artery. When the patients obtained steady state after about 5 min exercise, expired air as collected for 3 min, and blood samples were again taken as described above.

Pulmonary blood flow was calculated according to the Fick principle. *Systemic blood flow* was calculated from the following formula.

$$\text{Systemic blood flow (l/min)} = \frac{\text{Oxygen consumption (ml/min)}}{\text{Oxygen content of systemic artery} - \text{oxygen content of caval veins (ml/l)}}$$

The mean value of oxygen saturation in the superior and inferior caval veins was taken as the caval vein oxygen saturation at rest. During exercise the caval vein oxygen saturation was calculated as 75% of the inferior caval vein and 25% of the saturation in the superior caval vein.

Pulmonary flow ratio (PFR) was calculated directly as pulmonary blood flow/systemic blood flow

Table I. *Haemodynamic data at rest and during exercise*

Case no.	Sex	Age (y.)	O_2-consumption (ml/min)	Heart rate	O_2-saturation SA	PA	SVC	IVC	Blood flow Pulmonary (l/min)	Systemic (l/min)	(l/min/m²)
1	34	♂	R 184	70	99	90	82	89	13.6	9.1	5.1
			E 468	96	99	77	80	67	13.5	10.4	
2	17	♂	R 249	80	97	89	71	80	15.2	5.7	3.4
			E 660	100	97	79	71	46	17.8	7.1	
3	16	♀	R 195	78	99	86	58	75	8.6	3.5	2.1
			E 747	114	97	68	58	57	14.6	10.8	
4	18	♂	R 226	88	99	89	76	89	13.8	8.4	5.0
			E 595	114	96	84	75	69	29.6	13.9	
5	43	♂	R 358	70	98	86	75	77	15.1	8.3	3.7
			E 810	110	95	76	68	51	17.1	10.3	
6	15	♂	R 261	80	98	92	73	73	26.1	6.2	3.8
			E 656	100	98	79	65	75	21.6	16.4	
7	50	♀	R 228	80	99	92	86	86	17.1	9.2	5.4
			E 533	112	98	82	85	55	21.1	9.8	
8	22	♀	R 182	80	99	90	75	82	14.1	6.2	3.7
			E 511	120	96	78	71	52	20.3	9.2	
9	16	♂	R 257	84	97	92	67	77	31.7	6.3	4.3
			E 806	125	94	78	57	19	30.1	7.3	
10	14	♂	R 150	90	97	90	72	71	14.0	3.9	3.2
			E 564	125	95	78	62	37	22.7	7.5	
11	48	♀	R 187	70	95	90	72	79	25.6	6.6	3.8
			E 588	108	96	85	70	53	35.4	10.0	
12	49	♀	R 161	55	95	88	69	71	16.0	4.7	3.1
			E 487	92	95	79	65	44	19.4	6.8	
13	34	♀	R 258	85	97	87	70	82	15.4	7.4	3.9
			E 601	120	95	77	74	58	18.3	10.0	
14	21	♂	R 211	82	99	92	75	79	15.6	5.0	2.9
			E 631	110	99	87	73	53	26.5	7.8	
15	52	♀	R 294	70	98	80	61	66	9.2	4.8	2.5
			E 604	91	94	56	57	39	9.0	6.8	
16	29	♀	R 184	90	97	86	63	74	13.0	5.0	3.2
			E 650	130	96	73	54	34	20.7	8.3	

SA = systemic artery PA = pulmonary artery SVC = superior vena cava, IVC = inferior vena cava, s/d = systolic/diastolic, R = rest, E = exercise

RESULTS

Table I shows the pulmonary and systemic blood flow pulmonary blood flow ratio and the size of the left-to-right shunt in the patients at rest and during exercise The arterial oxygen saturation was normal in all cases at rest and on exercise, so there were no significant right-to-left shunts.

Table I presents further the pressures and the values of calculated pulmonary and systemic arteriolar resistance (PAR and SAR) In 14 patients the systolic pulmonary arterial pressure was below 35 mmHg at rest, but in 4 of these a slight elevation of the systolic pulmonary arterial pressure was measured during exercise. In patients nos. 1 and 15 the systolic pulmonary arterial pressure at rest was raised, and there was a slight increase in PAR. In the majority of the patients an insignificant systolic pressure gradient across the pulmonary valve was found

In all patients with the exception of nos. 3 and 15 the systemic blood flow was of normal

PFR	Left-to-right shunt (l/min)	Pressure (mmHg) PA (s/d)	SA (s/d)	PAR (dynes sec cm^{-5})	SAR (dynes sec cm^{-5})
1.5	4.5	48/20	108/68	147	661
1.3	3.1	76/36	140/80	280	738
2.7	9.5	10/4	88/60	26	982
2.5	10.7	22/8	96/52	35	789
2.5	5.1	17/3	76/44	47	1 326
1.3	3.8	24/4	128/68		681
1.6	5.4	29/2	132/72	49	914
2.1	15.7	27/7	156/76		633
1.8	6.8	22/8	132/64	96	867
1.6	6.8	44/22	160/100		963
4.2	19.9	24/3	148/80	37	1 394
1.3	5.2	31/6	136/64	44	469
1.9	7.9	34/10	176/100	80	1 113
2.2	11.8	64/14	172/108	119	1 061
2.3	7.9	25/7	120/72	63	1 160
2.2	11.0	30/10	148/72		904
5.0	25.4	32/7	120/88	13	1 244
4.1	22.8	50/14	136/84	15	1 162
3.6	11.1	17/5	96/60	28	1 470
3.0	15.2	35/12	112/60		852
3.9	19.0	14/2	128/64	6	1 090
3.5	25.4	29/1	148/68		800
2.4	11.3	29/2	96/44	30	1 090
2.9	12.6	56/14	148/64	66	1 176
2.1	8.0	17/7	128/76	47	1 038
1.8	8.3	27/9	140/88	44	864
3.2	10.6	27/5	112/64	31	1 280
3.4	18.7	32/7	128/60		924
1.9	4.4	74/18	140/64	304	1 600
1.3	2.2	104/28	144/64		1 176
2.6	8.0	14/4	96/64	25	1 152
2.5	12.4	25/8	[illegible]/60		790

magnitude at rest. It is remarkable that so many of the patients showed so slight an increase in the systemic flow during exercise. All patients except three had a fall of SAR during work. From one patient to another there was a considerable variation in pulmonary blood flow at rest (range 8.6–31.7 l/min). During exercise the pulmonary blood flow increased in most of the patients (Fig. 1) but was unchanged or slightly decreased in some cases. The absolute left-to-right shunt fell in 5 patients, was unchanged in 1 and moderately increased in 10 patients. No correlation was found between the magnitude of the shunt and the variation in the pulmonary blood flow during exercise. PFR varied at rest from 1.5 to 5. During exercise PFR fell in 13 and increased in 3 patients (Fig. 2).

The two patients with elevated resting pulmonary arterial pressure had a small left-to-right shunt at rest. In both of them the shunt was reduced during exercise. Of the 4 patients with increased systolic pulmonary arterial pressure on exercise one had a reduced shunt, one an unchanged and two an shunt du
ercise. In 3 of
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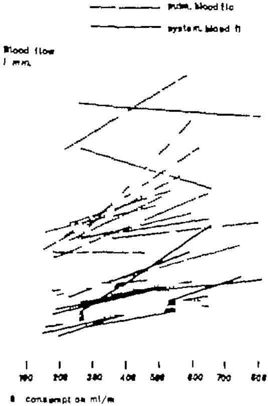


Fig 1 Pulmonary and systemic blood flow at rest and on exercise.

DISCUSSION

The difficulties in obtaining accurate flow measurements in patients with left-to-right shunts are well known, particularly in cases with ASD. The pulmonary arterial oxygen saturation is high, and there is a low arteriovenous oxygen difference. Small errors in measurement of the oxygen saturation may lead to large errors in calculated flow by the Fick principle. In ASD it is very difficult or impossible to obtain a truly mixed venous sample. In this study the average of the oxygen saturation values in the inferior and superior caval veins was taken as mixed venous blood oxygen saturation at rest. During exercise 75% of the saturation in the inferior and 25% in the superior caval vein was taken as mixed venous blood saturation according to Strøde Nielsen and Fabricius (8). It is, however difficult to obtain a truly mixed sample of blood from the inferior caval vein. On the one hand, the increased venous return from the working muscles of the legs leads to a reduction of the oxygen saturation. On the other hand the blood in the inferior caval vein, both during exercise and at rest, is more or less, and beyond control, contaminated by renal venous blood, which contributes to a too high estimate of oxygen saturation.

In agreement with others (2, 6, 7) the present study has shown that the systemic blood flow varies widely at rest in ASD. The results in this study cannot, however, be directly compared with those of other authors, since mixed venous blood has been estimated in different ways by different authors. Storstein and Efskind (6) used the oxygen saturation in the superior caval vein as the oxygen saturation of mixed venous blood. Davies and Gazetopoulos (2) took 2/3 of the oxygen saturation in the inferior and 1/3 in the superior caval vein as a rough approximation of oxygen saturation of mixed venous blood. Swan et al. (9) and Strøde Nielsen and Fabricius (7), like the authors of the present study calculated the average of the oxygen saturation in the inferior and superior caval veins as the mixed venous oxygen saturation at rest. During exercise the difficulty in comparing the shunt from one patient to another is even greater due to the unknown and highly varying degree of the venous flow from the working muscles. Davies and Gazetopoulos (2) and Swan et al. (9) used the same approximation for oxygen saturation in mixed venous blood on exercise as during rest, whereas Strøde Nielsen and Fabricius (7) used the same calculation as performed in this study. They found

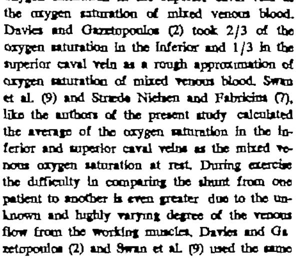


Fig 2 Pulmonary flow ratio at rest (left) and on exercise (right).

a decrease of the shunt in 11 patients, a doubtful increase in two patients with the smallest shunts, whereas PFR fell in all patients. These findings have not been confirmed in this study. The present investigation revealed no constant changes in the left-to-right shunt during exercise on a bicycle ergometer with a load from 150 to 200 kpm/min. An explanation of why the shunt was reduced in some patients and increased in others cannot easily be given.

It is a striking fact that so many of the patients show such a slight increase of the shunt during exercise loads giving the most significant rise in oxygen consumption.

Furthermore, it should be stressed that the PFR decreased during exercise in 13 of the total number of 16 patients.

The working load in the study was moderate, on the level of conventional clinical testing. Apparently it has been too small for a reliable evaluation of haemodynamics during submaximal and maximal exercise. The study seems, however, to *indicate that patients with uncomplicated ASD* tolerate major degrees of activity because the relative size of pulmonary blood flow tends to decrease during exercise. The validity of this conclusion is, however, restricted by the inaccuracy of methods in measuring the exact left-to-right shunt, particularly during physical activity.

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AZATHIOPRINE (IMUREL®) THERAPY IN CROHN'S DISEASE

Olav Fausa and Egil Gjone

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Abstract. Nine patients with Crohn's disease have been treated with azathioprine for periods ranging from 3 ½ to 12 months. The majority of them had postoperative recurrences, and all had extensive lesions. Treatment was followed by improvement in seven patients. Healing of fistulae occurred in two of three cases. It is suggested that azathioprine may be useful in treatment of selected cases of Crohn's disease

Crohn's disease with extensive lesions, postoperative recurrences or fistulae represents a difficult therapeutic problem. Failure of conventional treatment and the possible role of immunological mechanisms have encouraged trials with immunosuppressive agents. A dramatic response to azathioprine has been reported in selected cases (1 3 4). Recently Brooke et al. (2) concluded that the introduction of azathioprine might mean a breakthrough in the treatment in Crohn's disease.

Our first year's experience with azathioprine (Imurel®) in nine selected cases of Crohn's disease is reported.

MATERIAL AND METHODS

Nine patients with active Crohn's disease for 1 ½ to 10 years have been treated (Table I). Six cases had recurrences after intestinal resections and suffered from diarrhoea, malabsorption and abdominal pains. Three cases had persistent fistulae and one patient suffered recurrent attacks of subileus due to multiple stenoses of the small intestine. Two patients were severely ill because of acute exacerbations and were not fit for surgery.

Standard treatment was 2 mg Imurel®/kg b.wt. for the first 2 to 3 weeks followed by maintenance dose of 1.5 to 2 mg/kg (75 to 150 mg/day), except for one patient who received 3 mg/kg. In six patients Imurel® was given in addition to corticosteroids as the only change in therapy. The treatment lasted for 3 ½ to 12 months. The drug as well tolerated in all cases without signs of bone marrow depression. One patient suffered increased loss of hair.

Two cases failed to improve. All of the remaining seven patients responded with relief of pains and diarrhoea and marked improvement in the general condition. One of these cases (no. 4) had remarkable response lasting for 6 months. He gained 50% in weight during the first 3 months. He suffered an acute relapse, and second intestinal resection and colectomy with ileostomy ere performed. In two cases a reduction of corticosteroid dose was obtained almost relapse. The patients gained eight and returned to ork. In no case was there any conclusive improvement of the malabsorption tests recorded, even when diarrhoea subsided. No improvement in the radiological appearance of the intestinal tract was seen. An ileo-vesical fistula healed in 3 months. A sigmoid-vaginal fistula was incompletely healed after 4 months, but small perianal fistula remained unchanged. The last two patients were on corticosteroid treatment. Rectal ulcers healed in tw cases. Despite the unchanged radiological findings one patient as completely relieved of the attacks of subileus. Six of the nine patients returned to ork.

DISCUSSION

The present small trial does not allow any definite conclusions because the disease is unpredictable with spontaneous remissions. In accord with the other trials reported it seems as though azathioprine, despite its side-effects, might be useful in the management of otherwise intractable cases of Crohn's disease. Immunosuppression obviously may have a profound effect upon the disease (2). Striking improvement with azathioprine for treatment of the initial attacks has also been reported, but controlled studies are lacking.

Although the preliminary reports are encouraging, the well known hazards of immunosuppression must always be recognized and azathioprine should not replace other forms of treatment but be restricted to selected, complicated cases unresponsive to other therapy (1 5). The drug may be helpful, either alone or as an adjunct to corti-

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Table I *Summary of results*

S – small intestine, L – large intestine.

				Treatment			Assessment				
					Imurel		Improvement of				
Case no.	Age (y)	Previous operation	Site of lesion	Predni-son	Dose (mg)	Duration (mo.)	General condition	Pain	Diarrhoea	Fistula healed	Weight (% gain)
1	34	+	S L		75	12	+	+	+	+	+20
2	33		S L	+	150–200	12	+	+	+		+5–10
3	17	+	S L	+	75–100	10	+	+	+		+10
4*	17	+	S L		100–75	7	+	+	+		+50 (+30)
5	24	+	S L	+	150	7	+	+	+		+20
6	54	+	S L	+	75	5	0	0	0		0
7	39	+	S L	+	150	4½	0	0	0	–	0
8	35		S L	+	100–150	3½	+	+	+	+?	+20
9	17		S L		100	3½	+	+	+		+30

* The initial improvement was maintained for six months. H then suffered an acute relapse.

costeroids, in recurrent Crohn's disease after previous surgery which precludes further intestinal resection or in cases of very extensive bowel lesions which are not suitable for surgery. Healing of fistulae has been reported by many authors (1 2, 3 4 5) Failures might be related to the simultaneous treatment with Prednison.

In our experience this treatment may be of value for improving the condition in seriously ill patients before surgery. Azathioprine should be considered in all cases with inoperable fistulae and other complications which are inappropriate to corticosteroids, e.g. amyloidosis.

Clinical criteria for the withdrawal of azathioprine therapy cannot be given. Immunosuppression is highly undesirable as long-term therapy because of the serious side-effects and should not be continued indefinitely.

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A CONTROLLED STUDY OF PERORAL GLUCOSE, INSULIN AND POTASSIUM TREATMENT IN MYOCARDIAL INFARCTION

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Abstract. A double blind trial has been carried out in order to evaluate the possible effect of peroral glucose/potassium and insulin treatment in patients with acute myocardial infarction. One hundred and two and 96 patients were allocated to the treatment and control groups respectively and treated for ten days. There was a statistically significant difference in arrhythmias and conduction disturbances in favour of the treated group whereas the mortality in the treatment group was not significantly lowered. An effect of the treatment in patients with previous myocardial infarction was also noted.

Despite the introduction of coronary care units, the mortality in acute myocardial infarction is still alarmingly high. Continuous monitoring shows that the majority of patients with myocardial infarction develop arrhythmias (11 13). Some of these are probably related to a transient electric instability which does not necessarily depend on the extent of the myocardial damage. To reduce this arrhythmic tendency several drugs have been introduced, though with limited success. Another approach is the so-called polarizing treatment introduced by Sodi-Pallares et al. (23), who in animal experiments showed that ischemia leads to a loss of the normal ionic gradient for potassium across the myocardial cell membrane, i.e. potassium leaves the cell (10 22, 24).

The polarizing treatment was introduced to counteract this potassium loss, thus probably reducing the tendency to arrhythmias. The treatment originally (23 24 25 26) consisted of infusion of 10% glucose in water with added 40 mEq of KCl and 20 I.U. of regular insulin/1000 ml. The preparation was given by slow i.v. infusion (40 to 60 drops/min) The treatment was given 1 2 or more times a week or continuously for 2 or 3 days or throughout a week up to a month. In more serious cases stronger solutions were used. However influenced by the findings of Bajusz and Selye (4), Sodi-Pallares and his group added the low sodium and high water diet to the treatment owing to the fact that any loss of sodium from the cell favours the entrance of potassium. Sodi-Pallares obtained encouraging results, though predominantly in animal experiments, whereas his clinical studies were small and made mainly under uncontrolled conditions.

Several other workers have investigated the clinical effects of the regimen. The first who conducted a controlled clinical trial was Mittra (14). He used an oral regimen of 240 g glucose and 52–78 mEq potassium daily for 14 days following the sequential analysis technique. Ten I.U. of insulin were given twice daily After 14 days the mortality rate among the 85 controls was 28.2% as compared with 11 7% in the treated group consisting of an identical number of patients. Mittra's studies seem to have been made under proper controlled conditions and the difference in mortality between the treatment and control group was significant at 5% level.

Fletcher et al. (8) made a double blind study with the intravenous regimen and were unable to demonstrate any effect. However the number of patients studied was small (11 deaths out of 80 patients studied) Pentecost et al. (19), in a relatively large controlled study of 200 patients with intravenous GIK, demonstrated no effect on mortality or on arrhythmia rate. In this trial, however the treatment was given for only 48 hours.

In smaller studies Paulley et al. (18) and Pilcher et al. (21) reported some reduction in fatality rate

Table I. *Survey of total number of patients and reason for exclusion*

	Treated	Controls
Total no.	132	135
Coronary heart disease without sign of recent myocardial infarction	20	25
Patients dying within 3 h	2	4
Diabetes discovered after admission	2	2
No coronary heart disease	6	8
Remaining for evaluation	102	96

whereas Malach (12) could not demonstrate any effect of the treatment on mortality Lundman and Orinius (11) reported reduction in the in cidence of arrhythmias. Surawicz (27) reviewed the literature on polarizing treatment and discussed the physiological basis of the treatment. He concluded that there was no clear evidence that the intracellular potassium could be re stored by GIK treatment. Surawicz conclusion is difficult to understand in the light of the physiological experiments of Regan et al. (22), Øye and Sinclair (28) Cherbakoff et al., Sodi-Pallares et al. (25) and others (5 7 9 10).

The British Medical Research Council per formed a multicentre controlled trial with oral glucose and potassium. The mortality rate at the 28th day in the treated group was 23.9% (98 of 410 patients) and in the control group 25.3% (109 of 430 patients). There was no dif ference in the incidence of cardiac arrhythmias

Table II. *Symptoms and signs on admission*

	Treated	Controls
No. of patients	102	96
Males	75	82
Females	27	14
Mean age (y.)	60.4	59.2
No. with first infarction	85	73
No. with previous infarction	17	23
Arrhythmias and conduction disturbances on ECG on admission	26	19
Mean of first SGOT	77	103
No. with hypertension (> 100 mmHg diastolic pressure)	20	15
Hours from infarction until treatment started	11.8	13.5
Mean serum cholesterol (mg/100 ml)	293	294
No. with leukocytosis on first day	46	47
Weight (kg)	70.8	71.2

and conduction disturbances in the two groups. Sodi-Pallares et al. (26) commented on this British trial as a good example of the confusion that exists in the report on polarizing treatment" At the same time Sodi-Pallares stressed the importance of not using the oral route of administration, of strict low sodium, high potassium diet, and of adequate water intake. However although he advocated the intravenous route, we chose the oral regimen owing to the result of Mittra, and also because of experimental data which suggested a greater endogenous insulin response to oral than to intravenous administration of glucose in patients with myocardial infarction (17 20)

METHODS

Patients below 75 years of age were included if history suggesting myocardial infarction during the last 48 hours was given and the ECG and the clinical findings supported the diagnosis. Previously known diabetics and patients who showed glucosuria or ketonuria on admission were excluded together with seriously ill cancer patients, uremics, and patients with mental disorders.

Myocardial infarction was defined according to standard rules including characteristic substernal pain, ECG changes, serum GOT elevation to 50 U or more, elevation f sedimentation rate, leucocytosis over 9 000 cells/mm³ a typical temperature curve, and a pericardial friction rub (6).

The patients were randomized according to precon structed random list including stratification of patients with arrhythmias and conduction disturbances on admission, i.e. premature ventricular beats with a frequency higher than 10% atrial fibrillation or flutter atrioventricular block I, II, III, sinoatrial block, sinus bradycardia with less than 45 beats/min, sinus tachycardia with more than 130 beats/min, and left bundle branch block.

During the first 10 months of the study these disturbances were diagnosed by pulse palpation 4 times/24 h, two of which with simultaneous heart auscultation, and ECG once a day During the last 14 months of the study all the patients had additional continuous ECG monitoring for at least 4 days. The fact that not all the patients in the study were continuously ECG-monitored was of course drawback. This drawback, however, was reduced by the double blind design of the trial.

The hospital pharmacy made juice containing 50 g glucose/100 ml, acid. citric. 0.5% apoch conc. 5% spir. conserv 0.6% The placebo group received juice containing sodium cyclamate 2 g/100 ml instead of glucose. Potassium was given as mixture containing 9.2 g kalii citratis/100 ml, acid. citric. 0.6% succus ribis nigri 22.2% saccharum 37.8% spir conc. 0.6% The controls were given the same solution without potassium. Two hundred grams glucose and 55 mEq potassium were given daily in four divided doses in the treatment group.

During preliminary study 16 I.U. of short acting

Insulin was given twice daily with the last dose at 9 p.m. to cover at least the first part of the night with insulin. This, however, resulted in a tendency to hypoglycemia. To avoid this hazard it was decided to use long acting insulin, 16 I.U. (Insulin-Retard, Leo), in one morning dose only. With this regimen and the above mentioned doses of glucose and potassium a study was made in 9 patients, estimating the fasting blood sugar level at 6.30 a.m. and the blood sugar at 12 a.m., 4 p.m. and 00.30 a.m. On this regimen no hypoglycemia was noted and it was therefore decided to use this insulin dose in the present study.

Placebo insulin was made by Novo. The two insulins and the four mixtures were coded at the pharmacy. The code was kept unknown to the clinicians until the trial was finished. In addition to the GIK treatment the patients of both groups received routine treatment including bed rest and oral anticoagulants. Oxygen therapy and salt restriction were given in the majority of cases. Digitalis, diuretics, antiarrhythmic drugs and pacemaker were used when needed. The GIK treatment lasted 10 days and during this period the previously described criteria for recording arrhythmias and conduction disturbances were used. From the 11th day the ECG was recorded when clinical examination revealed arrhythmias or if a reinfarction was suspected.

MATERIAL

After 2 years 267 patients had been admitted to the trial, 132 in the treatment group and 135 in the control group. Of this number 30 treated patients and 39 controls (25% of all) had to be excluded for various reasons (Table I), the most important being coronary heart disease without definite signs of myocardial infarction according to the criteria.

One hundred and two treated patients and 96 controls remained for evaluation. Table II provides information as to the comparability of the two series. A moderate sex difference is noted and there are minor differences with respect to previous infarction, hypertension and mean SGOT level on admission. Twenty-six treated patients and 19 controls showed arrhythmias and conduction disturbances on ECG on admission. These rather small differences should give no reason to believe that the two groups were prognostically different on admission. Further comparability studies demonstrated no difference between the mean thrombotest values in the two groups during the stay in hospital. It is also of importance for comparability to know how many patients in the trial did not complete the treatment. Table III shows that in 4 and 10 patients in the treated and the controlled series, respectively, the treatment had to be finished before the intended 10 days, and the Table also shows the reason for this.

RESULTS

Table IV shows clinical and biochemical data during the total observation period, excluding number of deaths and arrhythmias. In all the respects presented the groups are quite similar.

Table V shows a difference in mortality between the two groups during the total observation period, i.e. during the stay in hospital. This difference is not of statistical significance ($p = 0.10$). During the treatment period, i.e. the first 10 days, the mortality was quite similar in the two series. From the 11th day 2 and 9 patients died in the treated and the control group respectively, the difference, however, not being statistically significant.

Table VI shows the number of sudden (unexpected) deaths in the two groups in relation to the number of patients who mainly died of shock and congestive failure. The difference (9 sudden deaths in the controls as opposed to 3 in the treated group) is not statistically significant.

Table VII shows that, among patients with previous myocardial infarction, 1 out of 17 (5.9%) died among the treated, as opposed to 9 out of 23 (39.1%) of the controls, a statis-

Table III. *Patients included in trial who did not complete GIK treatment*

	Treated		Controls	
	No. of pats.	Days (mean)	No. of pats.	Days (mean)
Nausea	4	4.2	6	5.4
Administrative failure	0		2	6.5
Severely ill	0		2	4.5
	4		10	

Table IV. *Clinical and biochemical data during total observation period*

	Treated	Controls
Typical ECG changes (%)	90.5	87.5
SGOT above 40 U (%)	95.7	91.3
SGOT above 200 U (%)	39.8	38.4
Sed. rate above 70 mm/h (%)	43.9	36.3
Congestive failure (%)	36.5	30.0
Shock (%)	6.6	3.6
Friction rub (%)	16.1	18.6
Pneumonia (%)	3.4	7.5
Thromboembolism (%)	2.0	[illegible]
Temp. above 38°C (%)	78.5	[illegible]
Heart volume (ml/m²)	470.0	[illegible]
Days in hospital (mean)	[illegible]	[illegible]

Table V *Mortality in the two series*

	Treated	Controls
No. of patients	102	96
Deaths		
First 10 days	7	7
From day 11	2	9
During total stay in hospital	9 (8.8 %)	16 (16.7 %)

Table VI *Causes of death during and after treatment in the two groups*

	Treated	Controls
Death mainly due to arrhythmia		
During treatment	3	2
After treatment	*0*	*5*
Myocardial rupture		
During treatment	0	1
After treatment	0	1
Shock/congestive failure		
During treatment	4	4
After treatment	2	3

tically significant difference, $p = 0.035$ Among patients with first myocardial infarction the deaths were equally distributed between the treated and the control group.

Table VIII shows the number of patients who developed arrhythmias and conduction disturbances without having this complication on admission. A highly significant difference in favour of the treated group is noted. ($p = 0.00007$) When studying the patients without arrhythmias or conduction disturbances in the first 24 hours, the difference in favour of the treated group is still present ($p = 0.0028$). Of those who developed arrhythmias 4 died among the treated and 3 in the control group.

Regarding the number of patients with arrhythmias and conduction disturbances present on admission, in whom these abnormalities later disappeared, there is no statistically significant difference between the groups.

Certain factors might possibly be unevenly distributed among the fatal treated and the fatal controls, such as age, hours from myocardial infarction until treatment started, hypertension, cholesterol level, nausea during stay and arrhythmias on admission but none of these factors turned out to be significantly unevenly distributed between the two groups.

The major side-effect of the treatment was nausea and a feeling of discomfort while taking the oral regimen This occurred in 31 patients in the control group and 21 in the treated. As mentioned in Table III, 6 controls and 4 treated patients refused further treatment *for this reason.*

DISCUSSION

This trial was performed in accordance with the double blind technique. It is, however still possible to raise some objections to the study The most important is maybe the fact that the treatment received by the control group was not inert i.e. the oral regimen in the control group theoretically might have been more harmful than no treatment at all The only side-effect noted, however was some nausea while taking the oral regimen (32 % in the controls and 21 % in the treated patients). Therefore an additional control

Table VII *Mortality within the two groups related to the occurrence of previous infarction*

	First MI		Previous MI	
	Total	Deaths	Total	Deaths
Treated	85	8 (9.4 %)	17	1 (5.9 %)
Controls	73	7 (9.6 %)	23	9 (39.1 %)

Table VIII *Recording of arrhythmias and conduction disturbances*

	Treated	Controls
No. of patients without arrhythmias or conduction disturbances on admission	76	77
No. of these developing arrhythmias and conduction disturbances during treatment ($p = 0.00007$)	23	49
No. of patients without arrhythmias or conduction disturbances in first 24 h	67	71
No. of these developing arrhythmias or conduction disturbances during treatment ($p = 0.0028$)	19	39
No. of patients with arrhythmias or conduction disturbances on admission, in whom these abnormalities disappeared during treatment	24	17

group with solely conventional treatment might have been desirable.

The remarkably low overall mortality in the series is probably related to the criteria of exclusion of diabetics and patients who were extremely ill or dying on admission.

The results show a difference in mortality during the stay in hospital in favour of the treated group, although not statistically significant. From the 11th day 2 died in the treated and 9 in the control group, i.e. after having completed the GIK regimen. One might therefore suspect a higher incidence of congestive failure or shock in the control group but Table IV demonstrates no evidence of this. Furthermore, the average relative heart weight of the fatal cases was 459 and 551 g in the treated and in the control groups respectively i.e. smaller hearts among the fatal treated cases.

There is also a difference in favour of the treated group with respect to sudden or unexpected death. The numbers, however are too small to justify any definite conclusion (Table VI).

The number of patients with arrhythmias and conduction disturbances present on admission, but later disappearing, was not significantly higher in the treated group, but the figures are rather small. However these abnormalities show a highly significantly lower rate in treated patients at risk, i.e. those not having arrhythmias or conduction disturbances on the first ECG or on the first day in hospital (Table VIII). Therefore, as regards arrhythmias and conduction disturbances, the findings in this study definitely favour the treated group.

The effect of the treatment in patients with previous myocardial infarction might support the assumption of other workers (1 24) who recommend GIK treatment in patients with advanced myocardial damage.

In most of the clinical trials with GIK treatment, only small numbers of patients have been studied or the studies have not been made under proper controlled conditions. Our results should preferably be compared with those of Mittra, Pentecost et al., and that of the British Medical Research Council. The first study of Mittra (14) seems very reliable, and the reduction in mortality rate in the treated group in comparison with the controls is significant at the 5% level. The study of the British Medical Research Council (13), which showed no effect of GIK, comprises a large number of patients and was carried out with oral regimen f r 14 days. This study was a multicentre trial and was composed of 13 smaller studies of which only one covered more than 100 patients. Furthermore, ECG was recorded only on admission and between days 3 and 5. This, of course, reduces the accuracy of registration of the arrhythmias and thereby the value of the trial. In addition the trial was not double blind. Mittra second study (16) was made with retrospective controls and does not fulfil the criteria of controlled conditions. Pentecost et al. (19) studied the effect of GIK in 200 patients with acute myocardial infarction and found no benefit from the treatment. Because of the short duration of the treatment (48 h) and the intravenous regimen, this study may be difficult to compare with ours.

Our study is therefore probably the largest one with the double blind technique. The difference between our findings and many others must be explained by methodological differences. The conclusion, therefore, might be that our study has demonstrated an effect of peroral GIK treatment used as a prophylactic measure in acute myocardial infarction. This effect is clearly demonstrated in arrhythmias and conduction disturbances, while the effect on the mortality rate is not statistically significant.

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PERSISTENT PARESES AFTER PORPHYRIC ATTACKS

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Abstract. During 25-year period 1942-1967 95 patients with acute porphyric attacks have been indexed in The Danish Porphyria Register. Fifty of these were abdominal, only 4 showed psychosis without pareses, and the remaining 41 were paralytic. Of the latter 17 died and 24 recovered, but 12 of the 24 exhibited residual pareses after three years. Of the patients with persistent pareses 50% were males as against only 24% of the total material. Contrary to the generally accepted view porphyric pareses cannot be regarded as essentially reversible. A substantial number of patients do not recover completely from their paralysis, phenomenon which seems to be more common in males than in females.

The pareses of acute porphyria are generally regarded as essentially reversible, a view recently clearly expressed in the monograph of Gajdos & Gajdos-Török (1) and in Ridley's (4) comprehensive survey of the neuropathology of acute intermittent porphyria. In two of Ridley's 25 cases of fully developed porphyric neuropathy the duration of the pareses was, however between four and six years, and Hotz (2) described a case of severe irreversible cerebral lesion following a single attack of acute porphyria in a 12-year-old girl, this patient was studied 25 years after her acute attack and still exhibited an organic psychosyndrome at that time. Further we found in our Danish material five cases of porphyric pareses of over ten years' duration. Consequently the present view that porphyric pareses are essentially reversible cannot be maintained.

MATERIAL AND METHODS

One of us has for 25 years studied porphyria in Denmark and registered the cases in The Danish Porphyria Register (6, 7 9). All Danish physicians are aware of this register in hich the great majority of patients with acute porphyria, if not all, are registered in the course of few years after their first attack. Further our systematic studies of these patients' families (7 9) is an effective support in obtaining complete coverage of all cases occurring in Denmark.

During the 25-year period 1942 1967 95 cases of acute intermittent porphyria or mixed porphyria were registered. Fifty of them (52.5%) had abdominal attacks without neuro-psychic involvement, only 4 (4.2%) exhibited psychoses without pareses, and the remaining 41 had more or less extensive pareses. Of the latter 17 (18%) died during the acute attack while 24 (25%) recovered. All these 24 cases had quadriplegia during the acute attack, and 12 of them still exhibited some degree of paresis after three years. These 12 cases are presented in Table I.

I all cases the diagnosis of porphyria was supported by urine analysis for porphyrins and porphobilinogen (PBG), and in most of them by analysis for delta-aminolevulinic acid (ALA) too (7 8). In some of the older cases analysis was performed in other laboratories, but in most the diagnosis was confirmed in our laboratory either in the patient or in relatives. Several of the patients had other clinically manifest cases of porphyria among their relatives. The method of family study is described previously (9).

DISCUSSION

As seen from Table I, 7 of the 12 cases with pareses of 3 years or more duration were males, which is remarkable, as only 24% of our total Danish material were males. The residual pareses varied from extensive lesions in all four extremities after 13 years (case 51) to partial paresis of one thumb after 17 years (case 123). In one case slow but complete recovery took place over 8 years (case 132), but in five pareses were still present 10 years or more after the tetraplegic attack (cases 24 51 91 107 and 123) and in two of these even after 25 years or more (cases 24 and 91).

One of our patients (case 105) exhibited what could be described as a chronic attack of acute

Table I. *Studies on 12 cases of residual pareses lasting 3 years or more after porphyric attack*

No. in Danish Porphyria Register	Sex	Date of birth	Occurrence of attack (y.)	Occurrence of attack (age)	Clinical features at attack	Nature of residual pareses	Year of study of residual pareses	Duration of pareses (y.)	Chemical data	Manifest cases in the family	Previous publications
24	♀	26.11.10	1944	34	Tetraplegic polyneuritis	Bilateral peroneal paresis (drop foot)	1970	26	UP much increased, PBG up to 44 mg/100 ml	0	Sonne (5) 1945
51	♂	21.08.31	1957	26	Severe tetraplegia with respiratory paralysis	Pronounced spastic pareses of both legs	1970	13	UP and PBG highly elevated during attack, PBG up to 30 mg/100 ml. 1967 PBG and ALA both elevated to about 3 mg/100 ml on several occasions	3	With (9) 1969 (family studies)
85	♂	31.03.07	1961	54	Tetraplegic polyneuritis	Still marked muscular atrophy of shoulder and biceps on both sides	1966	5	Increased UP in acute stage, no determination of PBG or ALA. 1962 fecal coproporphyrin 105 and protoporphyrin 250 µg/g dry matter	0	—
	♀	30.05.13	1958	43	Tetraplegic polyneuritis	Slight diffuse pareses of both arms and legs	1967	5	Increased UP during attack. Studies in our laboratory 1962 PBG 2.6 mg/100 ml positive screening test for porphyrin	0	—
	♀	19.09.07	1945	38	Tetraplegic polyneuritis	Paresis of both thumbs with atrophy of thenar muscles. Bilateral peroneal paralysis (drop foot)	1970	25	Increased UP during attack. Later numerous determinations in our laboratory e.g. PBG 6.8 mg/100 ml ALA 2.9 mg/100 ml 1970	2	Møller & Thomsen (3) 1949 With (7) 1963 (family studies)
100	♂	20.12.18	1963	45	Tetraplegic polyneuritis	Extensive pareses of all four extremities. Died in new attack 1967	1967	4	PBG 4.8 and ALA 4.1 during decline of acute phase	0 (among 7 relatives 3 carriers were found)	—

Table I (*continued*)

No. in Danish Porphyria Register	Sex	Date of birth	Occurrence of attack (y.)	Occurrence of attack (age)	Clinical features at attack	Nature of residual pareses	Year of study of residual pareses	Duration of pareses (y.)	Chemical data	Manifest cases in the family	Previous publications
105	♀	10.06.44	1964	20	Tetraplegia with respiratory paralysis	Prolonged activity of porphyria "permanent attack" with varying degree of paresis. Still tetraplegia although some amelioration	1970	6	Increased UP positive PBG during initial attack. 1964 PBG 8.8 mg/100 ml, 1968 PBG 3.0–8.0, ALA 2.0–6.5 in our laboratory	0 (10 relatives studied)	—
107	♀	08.10.27	1954	27	Tetraplegic polyneuritis	Atrophy of thenar and the interosseous musculature on both hands	1965	11	Increased UP positive PBG test during the attack (Medicinsk laboratorium, Copenhagen). No studies in our laboratory	4	With (9) 1969 (family study)
120	♂	13.04.09	1953	44	Tetraplegic polyneuritis	Defective extension of both wrists and the four ulnar fingers on both hands	1959	6	Increased UP and positive PBG. No studies in our laboratory	4	
123	♂	10.11.09	1948	39	Tetraplegic polyneuritis	Defective extension of thumb of right hand with atrophy of thenar and first interosseal muscle	1965	17	Increased UP and positive PBG during attack 1965 PBG 1.75 and ALA 1.25 mg/100 ml	1	
132	♂	04.03.12	1946	34	Tetraplegic polyneuritis	Gradual decrease of pareses in 8 years. Invalid pension, but finally completely restituted	1954	8	Increased UP and positive PBG during attack. No studies in our laboratory	4	With (9) 1969 (family study)
136	♂	12.08.41	1967	26	Tetraplegic polyneuritis with respiratory paralyses and cardiac arrest	Still pareses of all 4 extremities although considerably improved	1970	3	No chemical studies during the attack because the diagnosis of porphyria was not considered, although the attack began as acute abdomen. Later on several occasions normal PBG, ALA and porphyrins	0 (among 21 relatives studied 13 carriers were found)	With (9) 1969 (family study)

porphyria, i.e. a series of frequently recurring attacks, one of which was not over before the other began, and a continuous high excretion of the porphyrin precursors. PBG and ALA were constantly increased for years in several of the cases, a phenomenon which is often seen in latent porphyria. We have recently observed for instance, a patient in perfect health with a PBG concentration as high as 30 mg/100 ml at the close of a pregnancy.

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LEFT VENTRICULAR FUNCTION IN ISCHEMIC HEART DISEASE

Angiocardiographic and Hemodynamic Studies

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Abstract. Cases with ischemic heart disease often have increased left ventricular (LV) volume, often with asymmetric changes in the form of local asynergic areas. It has been known for several years that angina pectoris is usually accompanied by increased LV filling pressure. The authors have studied the relationship between the LV filling pressure and changes in the left ventriculogram in 34 cases with coronary artery disease. The left atrial (LA) mean pressure was used as an index of the LV filling pressure and was studied at rest and, in 26 cases, also during supine work. Coronary arteriographies were performed in all cases, as well as LV cineangiography in at least one plane. Though admittedly small, the groups were comparable with regard to age and cardiac index at rest. An increased filling pressure at work load which caused anginal pain was mainly seen in the groups with increased LV end-diastolic volume, and there was no difference between cases with and without local asynergy

In a previous communication (2) some correlations between findings at left ventricular (LV) cineangiocardiography coronary arteriography and work test in patients with angina pectoris were studied.

A report of similar studies of the correlations between findings at LV cineangiocardiography coronary arteriography and hemodynamic studies of the function of the left ventricle at rest and during exercise in patients with ischemic heart disease (IHD) is here presented.

MATERIAL

The material consisted of 34 patients, 3 women and 31 men. Mean age for the patients was 53.3 years (range 37–65). All patients had symptoms and signs of IHD The ECG showed healed myocardial infarction in 10 cases. Regular sinus rhythm was present in all cases. Twenty patients had retired from their ordinary work because of the disease, two were working half-time and 12 full-time. All patients were referred to our hospital for preoperative evaluation of IHD. There as no further selection of patients.

METHODS

The LA mean pressure at rest in supine position was measured in all cases; LA pressure at exercise in supine position was determined in 26. The work load during exercise varied from case to case (75–600 kpm/min) and was chosen with previous work test (16) in sitting position as guide. The work continued until the patients developed angina pectoris. In one patient with pressure rise to 22 mmHg no pain occurred. The LA pressure was determined with transseptally introduced teflon catheter pressure transducer (type EMT 34–35) and direct writing jet recorder (Mingograph 81). In 77 patients the cardiac output at rest was determined using the Fick principle.

Cineangiocardiography of the LV was performed in all patients. Approximately 0.5 ml/kg b.wt. of Isopaque® 350 (methylglucamine metrizoate with small amount of calcium and magnesium metrizoate added, Nyegaard, Oslo) was injected. The injections were made in the LA through the transseptally introduced catheter in most cases. In 5 cases the injections were made through grey Ödman-Ledin catheter percutaneously introduced from the femoral artery into the LV. The rate of injection varied from 20–30 ml/sec. Cineangiocardiograms of the LV were obtained with the patient in right anterior oblique position. Care was taken to have the largest projection of the LV perpendicular to the central X-ray beam. The angiocardiographies were monitored by TV fluoroscopy and also recorded on videotape. The exposures are registered on continuous ECG. Only normally conducted beats are considered for LV evaluation. In most patients only one injection was performed. In 7 patients with pathological changes in the left ventricle second injection with the patient in a different position was made, allowing approximately 10 min between the injections.

Coronary angiography was in most cases performed selectively by the technique described by Judkins (12) 3–4 ml of contrast medium was injected manually into each coronary artery Cineradiographic recordings in one

porphyria, i.e. a series of frequently recurring attacks, one of which was not over before the other began, and a continuous high excretion of the porphyrin precursors. PBG and ALA were constantly increased for years in several of the cases, a phenomenon which is often seen in latent porphyria. We have recently observed for instance, a patient in perfect health with a PBG concentration as high as 30 mg/100 ml at the close of a pregnancy

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results from the nature of the sample. Patients referred for preoperative evaluation of angina pectoris will as a rule not be in heart failure. A lower cardiac output in cases with local asynergy in the left ventricle, both when compared with normal controls and with cases with IHD without asynergy has been reported (10). This probably reflects rather the inclusion of cases with heart failure than an effect of the asynergy per se. Also differences in age might explain part of the difference between their findings and ours, as well as the limited number of patients in our series.

The different forms of asynergy described by Herman et al. (10) were also seen in our patients, with the exception of "asynchrony". We have not been able to recognize this particular form of asynergy in this or other groups of patients with IHD. Subdivision of the patients according to different types of asynergy was not applied since there were only 17 patients with asynergy in our material. The validity of findings at left ventricular cineangiography in one plane, particularly for the diagnosis and measuring of the size of an asynergic part of the wall, has been discussed (1). It is clear that a biplane technique would be superior to single plane in this context. However the limitations of the single plane technique may be overcome to a certain extent by the use of videotape recording, which permits instant evaluation of the angiographic result. A second angiocardiography can then be performed with the left ventricle in a different projection, more favourable for diagnosis of the size and location of an asynergic part of the wall.

In conclusion, it may be stated that a patient with an angiographically normal LV will as a rule have a normal LV filling pressure at work in spite of angina pectoris. Patients with increased LV volumes will as a rule develop high LV filling pressure at work. Within this last group the presence and the size of an asynergic part of the left ventricular wall will have only little influence on the LA pressure at work.

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THE NATRIURETIC EFFECT ADDITION OF QUINETHAZONE AND FUROSEMIDE IN CONGESTIVE HEART FAILURE

A Permutation Trial Test

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Abstract. The combined natriuretic effect of quinethazone and furosemide has been compared to the action of single drugs in 12 patients with congestive heart failure using a permutation trial test. The results indicate that the combined effects of the drugs represent an effect addition of two diuretics which act by different mechanisms.

In previous studies it has been shown that in patients with congestive heart failure the saluretic effect of the combination of quinethazone (2-ethyl-7-chloro-6-sulfamyl 1,... dihydro-4-quinazolone) (1 5 8, 9 12, 13 15) and furosemide (4-chloro-N (2 furylmethyl) 5-sulfamoyl-anthranilic acid) (1–4 7 9 10, 14 16) tended to be superior to that of double doses of the single drugs (9). While the results indicated that the combined administration of these diuretics might be useful in the treatment of advanced heart failure the design of the experiments did not permit definite conclusions concerning the nature of their combined effects. Therefore, an extension of the study is desirable in order to define the mechanism of their interaction.

For this purpose the present study was designed to compare the effects of the single agents with the combined effects of the drugs at the same dose levels. The experiments were performed as a permutation trial test which provides, for all diuretic treatments, an equal chance of showing their effects regardless of the variations caused by different patients and by varying sequence of administration of drugs (9).

MATERIAL AND METHODS

The patients were adult subjects with organic heart disease of rheumatic, arteriosclerotic or congenital origin and signs of congestive heart failure. All patients had previously received diuretic treatment which was discontinued at the start of the study. Digitalis treatment when given previously was continued during the trial.

The subjects received a sodium-poor diet, 5 g of sodium chloride and 3 g of potassium chloride daily during the week of study. The fluid intake was fixed at 000 ml day.

The urine was collected in 24-hour periods, and urinary excretion of water, sodium, chloride, potassium and creatinine, osmolal clearance and free water clearance were determined according to methods previously described (9). Body weight was measured every morning in the fasting state.

The administration of diuretics which took place on the fourth, fifth and sixth day of the week of trial followed the scheme shown in Table I, where A, B and C indicate different types of diuretic regimens. The trial included 12 patients and compared the effects of quinethazone 50 mg (A), furosemide 40 mg (B) and the combination of quinethazone 50 mg + furosemide 40 mg (C). All drugs were given orally at 8 a.m. A random allocation of patients to treatment programmes was secured.

The statistical analysis takes advantage of the design of the experiment, which takes into account variations due to different patients and to different sequence of administration of drugs. Through analysis of variance the error variance is reduced and the significance of the effects of the treatments alone is increased. The effects of treatments may be resolved into two comparisons by means of the rules of orthogonality (9). The first comparison is between two groups of treatments, and the second is between the sum of these two treatments and the third treatment.

It is a prerequisite for the design of the study that the action of the diuretics has ceased before the administration of the next treatment group. Available reports appear to indicate that this holds true for quinethazone (8, 12, 15) as well as for furosemide (1, 14).

Table I. *Sequence of administration of diuretic treatments*

A, B, C, see text

No. of pats.	Days 1	2	3
2	A	B	C
2	A	C	B
2	B	A	C
2	B	C	A
2	C	A	B
2	C	B	A

RESULTS

The combination of diuretics compared to single drugs

The sodium, chloride, potassium and water outputs and the osmolal clearance were significantly higher after the combination of drugs (C) than after any of the single diuretics (A or B) as shown by the comparisons (A − C), (B − C) and (A + B − 2C) ($p < 0.01$)

Correspondingly the b.wt. showed the largest change after the combined drugs. The changes were: +0.13 kg after quinethazone, +0.05 kg after furosemide and −0.55 kg after the combination of drugs. The comparisons (A − C) and (A + B − 2C) were significantly different ($p < 0.05$).

As shown in Fig. 1 and Table II the mean value for sodium excretion was 169 mEq after the combination of drugs, 93 mEq after quinethazone and 72 mEq after furosemide. Similarly the mean value for chloride output was 222 mEq after the combination of drugs, 116 mEq after quinethazone and 93 mEq after furosemide. For both parameters the effect of the combination of drugs represents slightly more than the sum of the responses to the single diuretics.

Comparison of quinethazone and furosemide

As shown in Fig. 1 and in Table II, there was no significant difference between the effects of quinethazone (A) and of furosemide (B) with regard to sodium, potassium, chloride and water excretion or osmolal clearance. The only difference was that the negative free water clearance, i.e. the tubular reabsorption of solute-free water was lower after furosemide than after quinethazone ($p < 0.05$), reflecting the specific inhibiting action of furosemide on sodium reabsorption in the ascending limb of Henle's loop with subsequent dissipation of renal concentrating capacity (3, 4, 10, 16)

Table II. *Statistical analysis of renal water, solute and electrolyte excretion*

A, B, C, see text

	Units	Mean 24-h values for each treatment: A	B	C	Variance ratios, Sources of variation: Patients	Days	Treatments	Comparisons: A − B	A − C	B − C	A + B − 2C
Significance limits	F 99				3.29	5.85	5.85	8.10	8.10	8.10	8.10
	F 95				2.31	3.49	3.49	4.35	4.35	4.35	4.35
Urine											
Sodium	mEq/24 h	93	72	169	3.13	3.20	13.75	1.19	15.17	24.88	26.30*
Potassium	mEq/24 h	88	82	112	3.54	0.39	8.06	0.61	9.12	14.46	15.52
Chloride	mEq/24 h	116	83	222	2.61	1.84	18.01	1.01	21.36	31.66	35.01
Osmolal clearance	ml/24 h	2 064	1 913	2 796	4.01	0.47	15.35	0.78	18.44	26.84	29.93
Free water clearance	ml/24 h	−990	−721	−910	5.07**	1.37	2.77	5.27	0.47	2.59	0.28
Diuresis	ml/24 h	1 073	1 192	1 887	2.36	3.00	19.94	0.72	34.20	24.97**	39.21**
Creatinine	mg/24 h	893	896	938	3.84**	0.03	0.25	0.00	0.41	0.35	0.51
Body weight	kg	0.18	0.05	−0.55	1.05	0.49	3.22	0.06	3.35	4.24	6.38

$p < 0.05$ $p < 0.01$, $p < 0.001$ Significance limits (99.9 %) for treatments 9.95 for comparisons 14.80.

DISCUSSION

Descriptions of the combined effects of drugs producing the same overt effect are intended to indicate whether they are equal to, or greater or less than, those expected by simple addition (6). However two types of additive behaviour can be distinguished. If the drugs are close congeners that act on the same receptors, doses of one drug should substitute for those of the other in proportion to their relative potency over a wide range of combinations. Only drugs that exhibit this dose addition are properly described as additive.

Deviations from dose addition are termed supra-additive or infra-additive. These latter descriptions imply that the drugs act by different mechanisms. If the combined effects of the drugs are equal to the sum of their individual effects they are said to exhibit effect addition or summation. Deviations from summation are usually described as supra-additive or infra-additive (6).

In the present study the combination of quinethazone and furosemide promoted a sodium and chloride excretion which was equal to or perhaps slightly larger than the algebraic sum of the effects of the single drugs as shown in Fig. 1 and in Table II. According to the definitions above, the combined effects of the drugs represent an effect addition or a summation.

Apparently the increased response to the combination of quinethazone and furosemide follows the pattern described for drugs acting by different mechanisms and cannot be explained simply as a result of an increased dose of one diuretic, acting through the same mechanism. Three lines of investigation support this interpretation. Firstly in our previous experience the combined effects *of the two drugs exceeded the responses to double* doses of the single drugs as shown in Fig. 2 (9). Secondly the dose response curves of quinethazone and of furosemide demonstrate that after a double dose the natriuresis is usually less than the sum of the responses to two single doses (2, 5 11 13 14). Thirdly clinical and laboratory studies indicate differences in mechanism of action between quinethazone and furosemide.

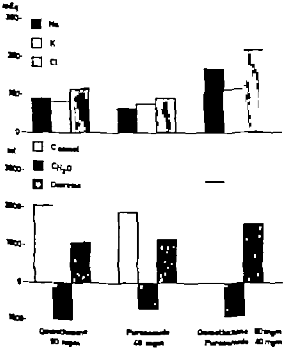


Fig. 1. Mean values for urinary electrolyte excretion, osmolal and free water clearance and diuresis in relation to diuretic treatments.

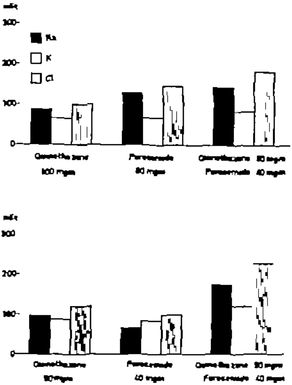


Fig. 2. Mean values for urinary electrolyte output after combination of drugs and after single and double doses of single diuretics.

Studies in man and in dogs have established that quinethazone is a saluretic agent with a potency similar to the benzothiadiazine diuretics and with a major site of action in the distal segment of the nephron (1 5 8 9 12, 13 15 17) while furosemide is a more potent saluretic drug which is able to inhibit renal tubular sodium reabsorption more extensively throughout the nephron, including the ascending limb of Henle s loop (1–4 7 9 11 14 16) This latter effect of furosemide is also apparent from the decrease of renal tubular reabsorption of solute-free water found in the present study

Apparently the combined effects of quinethazone and furosemide at low dose levels may be described simply as an effect addition or a summation produced by two drugs acting at least in part through different mechanisms or at different sites in the nephron. However the administration of a small dose of quinethazone to patients receiving long-term treatment with larger doses of furosemide and spironolactone results frequently in a very marked natriuresis. This type of response fulfils the criteria of a supra-additive effect addition and reflects a more complex mechanism of interaction of diuretics. This type of effect addition is discussed in a following paper (10)

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THE SUPRA ADDITIVE NATRIURETIC EFFECT ADDITION OF QUINETHAZONE OR BENDROFLUMETHIAZIDE DURING LONG-TERM TREATMENT WITH FUROSEMIDE AND SPIRONOLACTONE

Permutation Trial Tests in Patients with Congestive Heart Failure

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Abstract. The additive natriuretic effects of single doses of quinethazone (50 mg) or bendroflumethiazide (5 mg) have been studied in patients with advanced congestive heart failure receiving longterm treatment with furosemide (160 mg) and spironolactone (100 mg) daily. Three permutation trial tests were performed. In the first trial including 12 patients a significant increase of renal sodium, chloride, potassium and water excretion and osmolal clearance was found after supplementary administration of quinethazone or bendroflumethiazide. In the second trial including 6 patients the response to supplementary quinethazone (50 mg) was definitely superior to that of additional furosemide (80 mg). Accordingly the response to supplementary quinethazone cannot be explained as dose addition effect of drugs acting on the same renal tubular receptors, but must represent an effect addition of diuretics acting *by different mechanisms or at different* sites in the nephron. The third trial compared in 6 patients the effects of quinethazone (50 mg) + spironolactone (100 mg), of furosemide (160 mg) + spironolactone (100 mg), and of quinethazone (50 mg) + furosemide (160 mg) + spironolactone (100 mg). In terms of natriuresis, chloruresis, cation excretion and weight loss the responses to the combination of all three drugs are significantly larger than the sums of the effects of other treatments. It is concluded that the combined effects of the drugs represent a supra-additive effect addition (or supra-additive summation). A tentative explanation of the mechanism of this effect in terms of inhibition of renal tubular sodium transport is given. Since the combined effects of all three drugs involve a tendency to development of hypokalaemia, hypochloraemia and alkalosis, it is recommended that the supplementary use of quinethazone or bendroflumethiazide in this setting is combined with the administration of potassium chloride.

The present study was prompted by the clinical observation *that patients with advanced heart* failure receiving long-term treatment with furosemide (4-chloro-N(2 furylmethyl)5-sulfamoyl-anthranilic acid) (1–4 8, 17 19 23) and spironolactone (3-(3-oxo-7 alpha-acetylthio-17 beta-hydroxy-4-androsten-17 alpha-yl) propionic acid gammalactone) (2, 5 11 15 20) often show a marked natriuresis after the supplementary administration of a small dose of quinethazone (2-ethyl-7-chloro-6-sulfamyl-1,2 dihydro-4-quinazolone) (6 9 18 19 28) or of bendroflumethiazide (3-benzyl-6-(trifluoromethyl)-7-sulfamyl-3 4-dihydro-,H 1,2,4-benzothiadiazine 1 1-dioxide) (5 10 12, 16, 24 26 28).

While in our previous experience the combined *effects of quinethazone and furosemide*, as compared to the actions of the single drugs, could be explained in terms of a simple effect addition or summation of two drugs acting by different mechanisms (18) the response to the supplementary *administration of quinethazone* or bendroflumethiazide to patients receiving long-term treatment with furosemide and spironolactone suggested a supra-additive effect addition.

The present study was designed to examine the effects of the supplementary use of quinethazone or bendroflumethiazide in this latter setting and to study the pattern of additive effect obtained by means of variations in treatment programmes. The study was performed as permutation trial tests in which, through statistical analysis, the error variance is reduced while permitting an evaluation of the drug effects separate from the influence *of different patients and varying sequence* of administration of drugs (13 18 19).

Table I. *Sequence of administration of diuretic treatments*

A, B, C, see text

No. of pats.		Days		
Trial 1	Trials 2 and 3	1	2	3
2	1	A	B	C
2	1	A	C	B
2	1	B	A	C
2	1	B	C	A
2	1	C	A	B
2	1	C	B	A

MATERIAL AND METHODS

The patients were adult subjects with organic heart diseases of arteriosclerotic, rheumatic or congenital aetiology and signs of congestive heart failure. All patients had received digoxin, furosemide 80 mg b.i.d. and spironolactone 25 mg q.i.d. for at least two weeks and required more intensive diuretic treatment.

The subjects were given a sodium-free diet and a supplement of 3 g of sodium chloride daily. The fluid intake was fixed at 1 500 ml/day.

Twentyfour-hour urines were collected from 7 a.m. to 7 a.m. and blood samples were taken and b.wt. measured in the fasting state. Serum and urine electrolytes and osmolal and free water clearances were determined as described in a previous paper (19).

During the week of trial the patients received digoxin, furosemide 80 mg b.i.d. and spironolactone 25 mg q.i.d., with one exception mentioned below. The supplementary diuretics were given on the second, fourth and sixth day of the trial according to the scheme shown in Table I. A random allocation of patients to treatment programmes was secured.

In the first trial, which comprised 12 patients, a comparison was made of the effect of the supplementary administration of quinethazone 50 mg (A), of placebo (B) and of bendroflumethiazide 5 mg (C).

In the second trial, which included 6 patients, the effect of supplementary quinethazone 50 mg (A) was compared to the actions of placebo (B) and of additional furosemide 80 mg (C).

The third trial, comprising 6 patients, was designed to examine the nature of interaction of the drugs. This trial compared the effects of quinethazone 50 mg + spironolactone 100 mg (A) with the effects of furosemide 160 mg + spironolactone 100 mg (B) and of quinethazone 50 mg + furosemide 160 mg + spironolactone 100 mg (C).

The statistical methods used have been described previously (13, 19, 22).

Dose addition describes the combined effects of two drugs acting on the same receptors if doses of one drug are able to substitute for those of the other in proportion to their relative potency. *Deviations* from dose addition are termed *supra-additive* or *infra-additive* and imply that the drugs act by different mechanisms. *Effect addition* or *summation* describes the combined effects of two drugs when the response is equal to the sum of their individual effects. *Deviations* from effect addition or summation are usually termed *supra-additive* or *infra-additive* (7).

Table II. *Statistical analysis of renal electrolyte, water and solute excretion in trial I*

A, B, C, see text

		Mean 4-h values for each treatment			Variance ratios — Sources of variation			Comparisons			
	Units	A	B	C	Patients	Days	Treatments	A−B	A−C	A+C−2B	B−C
Significance limits	F 99				3.29	5.85	5.85	8.10	8.10	8.10	8.10
	F 95				2.31	3.49	3.49	4.35	4.35	4.35	4.35
Urinary excretion											
Sodium	mEq/24 h	170	55	159	1.34	1.74	10.18[b]	16.69[b]	0.14	20.21[b]	13.71[a]
Potassium	mEq/24 h	150	89	140	8.50[a]	0.78	8.24[a]	14.21	0.33	16.16[a]	10.19[a]
Chloride	mEq/24 h	269	87	249	2.31	0.80	10.80[b]	18.00[b]	0.22	21.39[a]	14.19[b]
Osmolal clearance	ml/24 h	3 133	2 191	3 339	5.73	0.13	7.38[a]	8.75[a]	0.41	14.35[a]	12.99[a]
Free water clearance	ml/24 h	−337	−359	−467	11.04[a]	1.92	0.87	0.04	1.32	0.22	1.00
Diuresis	ml/24 h	2 879	1 853	2 923	3.38	0.30	8.76[a]	12.59[a]	0.02	17.51[b]	13.68[a]
Creatinine	mg/24 h	934	927	953	7.73	1.60	0.05	0.00	0.05	0.05	0.11
Body weight	kg	−0.80	0.48	−0.70	0.74	1.24	9.95[a]	16.14	0.11	19.75[a]	13.54[a]

[a] $p < 0.01$ [b] $p < 0.001$. Significance limits (99.9 %) for treatments 9.95, for comparisons 14.80.

Table III *Statistical analysis of serum electrolyte and creatinine changes in trial 1*

A, B, C, see text

		Mean 24-h values for each treatment			Variance ratios						
					Sources of variation			Comparisons			
	Units	A	B	C	Patients	Days	Treatments	A − B	A − C	A + C − 2B	B − C
Significance limits	F 99				3.29	5.85	5.85	8.10	8.10	8.10	8.10
	F 95				2.31	3.49	3.49	4.35	4.35	4.35	4.35
Serum osmolality	mOsm/kg	−1.58	0.25	0.16	0.59	0.24	0.75	1.18	1.08	0.43	0.00
Serum sodium	mEq/l	−0.08	−0.41	−0.08	1.19	0.91	0.08	0.07	0.01	0.14	0.16
Serum potassium	mEq/l	−0.32	0.49	−0.27	0.72	3.28	4.82[a]	7.69[a]	0.01	9.63[b]	6.76[a]
Serum chloride	mEq/l	−3.25	1.75	−2.66	0.68	1.71	8.00[b]	13.38[b]	0.18	15.81	10.44[b]
Serum standard bicarbonate	mEq/l	0.20	−0.83	1.23	0.86	7.65	4.55[a]	2.38	2.16	6.94[a]	9.11
Serum creatinine	mg %	0.04	−0.07	0.01	0.33	2.66	1.33	2.66	0.00	3.00	1.66

[a] $p<0.05$, [b] $p<0.01$ $p<0.001$. Significance limits (99.9 %) for treatments 9.95, for comparisons 14.80.

RESULTS

Trial 1

This trial compared the effects of supplementary administration of quinethazone 50 mg (A), of placebo (B) and of bendroflumethiazide 5 mg (C). The results are shown in Tables II and III and in Fig. 1

1 Quinethazone compared to placebo (comparison A − B). The mean values for renal output of sodium, chloride, potassium, water and osmolal clearance were significantly higher after quinethazone (A) than after placebo (B) ($p < 0.01$). Similarly b.wt. decreased significantly after quinethazone ($p < 0.001$), indicating the saluretic-diuretic effect of this drug when given as supplement to patients receiving furosemide and spironolactone as long-term treatment.

2 Bendroflumethiazide compared to placebo (comparison B − C) Similarly to the findings above, the renal excretion of sodium, potassium, chloride, water and osmolal clearance were significantly higher after bendroflumethiazide (C) than after placebo (B) ($p < 0.01$). A significant decrease in b.wt. confirmed the saluretic effect of bendroflumethiazide in this setting.

The comparison (A + C − 2B) confirms the pattern found for the single diuretics compared to placebo as shown in Table II.



Fig. 1 Mean values for urinary excretion, osmolal clearance, free water clearance and diuresis in relation to supplementary diuretic treatments in trial 1.

Table IV. *Statistical analysis of renal electrolyte, water and solute excretion in trial 2*

A, B, C, see text

	Units	Mean 24-h values for each treatment: A	B	C	Variance ratios — Sources of variation: Patients	Days	Treatments	Comparisons: A − B	A − C	B − C	B + C − 2A
Significance limits	F 99				6.63	8.65	8.65	11.26	11.26	11.26	11.26
	F 95				3.69	4.46	4.46	5.32	5.32	5.32	5.32
Urinary excretion											
Sodium	*mEq/24 h*	*177*	*46*	*59*	*1.63*	*1.07*	*5.20*[a]	*8.56*	*6.95*[a]	*0.08*	*10.32*[a]
Potassium	mEq/24 h	106	63	62	0.43	0.66	2.65	3.82	4.12	0.00	5.29
Chloride	mEq/24 h	240	58	73	0.85	0.33	6.14[a]	9.97[a]	8.39[a]	0.06	12.22[b]
Osmolal clearance	ml/24 h	3 211	1 806	1 829	0.35	0.45	3.82	5.83	5.64[a]	0.00	7.65[a]
Free water clearance	ml/24 h	−678	−314	−362	1.76	1.06	2.14	3.64	2.75	0.06	4.23
Diuresis	ml/24 h	2 533	1 492	1 467	0.84	0.33	3.20	4.69	4.92	0.00	6.41
Creatinine	mg/24 h	967	830	720	0.49	0.27	0.76	0.46	1.51	0.29	1.22
Body weight	kg	−1.08	0.26	−0.13	0.66	0.15	2.50	4.74	2.34	0.41	4.60

[a] $p < 0.05$, [b] $p < 0.01$

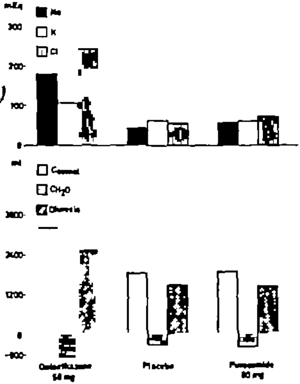


Fig. 2. Mean values for urinary electrolyte excretion, osmolal clearance, free water clearance and diuresis in relation to supplementary diuretic treatments in trial 2.

3. Quinethazone compared to bendroflumethiazide (comparison A − C). As shown in Table II and in Fig. 1, no significant differences were found between the effects of 50 mg of quinethazone and of 5 mg of bendroflumethiazide.

4. Serum electrolyte changes. It appears from Table III that both supplementary diuretics induced a significant tendency to development of hypokalaemia, hypochloraemia and metabolic alkalosis, while serum osmolality and serum sodium concentrations remained unchanged. Serum creatinine levels were unaffected.

Trial 2

This part of the study compared the effects of *supplementary administration of quinethazone* 50 mg (A) of placebo (B) and of additional furosemide 80 mg (C). The results are shown in Table IV and Fig. 2.

1. Quinethazone compared to placebo (comparison A − B). As shown in Table IV and in Fig. 2, the renal output of sodium and chloride and osmolal clearance were significantly higher after quinethazone (A) than after placebo (B), although the level of statistical significance is lower because of the smaller series ($p < 0.05$). The renal excretion of potassium and water and the change of b.wt. showed a similar trend.

2. Quinethazone compared to additional furo-

Table V *Statistical analysis of renal water solute and electrolyte excretion in trial 3*

A, B, C, see text

	Units	Mean 24-h values for each treatment			Variance ratios: Sources of variation			Comparisons			
		A	B	C	Patients	Days	Treatments	A−B	A−C	B−C	A+B 2C
Significance	F 99				6.63	8.65	8.65	11.26	11.26	11.26	11.26
limits	F 95				3.69	4.46	4.46	5.32	5.32	5.32	5.32
Renal output											
Sodium	mEq/24 h	53	89	265	4.20[a]	2.83	16.76[b]	0.86	29.33[c]	20.10[b]	32.66[c]
Potassium	mEq/24 h	64	55	89	5.78[a]	0.10	4.72[a]	0.53	4.91	8.70[a]	8.90[a]
Chloride	mEq/24 h	50	107	264	4.71	3.07	20.23[c]	2.64	37.69[c]	20.37[b]	37.83[c]
Osmolal clearance	ml/24 h	1 609	1 844	3 194	6.52[a]	1.84	10.49[b]	0.39	18.01	13.06	20.58
Free water clearance	ml/24 h	−742	−586	−761	3.40	0.64	0.43	0.57	0.00	0.72	0.86
Diuresis	ml/24 h	867	1 258	2 433	7.40	2.31	25.18[c]	2.90	46.49[c]	26.15[c]	47.46[c]
Body weight	kg	+0.30	+0.16	−1.26	2.27	3.18	13.69[b]	0.15	22.30[b]	18.66	27.27[c]

$p<0.05$, $p<0.01$ $p<0.001$ Significance limits (99.9 %) for treatments 18.5 for comparisons 25.40

semide (comparison A−C). Similarly to the findings above, the renal excretion of sodium, chloride and osmolal clearance were significantly higher after quinethazone (A) than after additional furosemide (C) ($p<0.05$). The renal output of potassium and water and the change of b.wt. showed a similar trend.

3 Additional furosemide compared to placebo (comparison B−C). Although the mean values for renal sodium and chloride excretion and osmolal clearance were slightly higher after additional furosemide than after placebo, significant differences did not occur

4 Quinethazone compared to other treatments (comparison B+C−2A). The comparison (B+C−2A) brings out clearly the superior effect of quinethazone as demonstrated by the significant differences in renal output of sodium, chloride and water and in osmolal clearance ($p<0.05$). This finding confirms that the natriuretic effect of supplementary administration of quinethazone to patients receiving long-term treatment with furosemide and spironolactone does not simply represent a dose addition effect, but rather an effect addition or summation (7 18).

Trial 3

This trial compared the effects of quinethazone 50 mg + spironolactone 100 mg (A) of furosemide 160 mg + spironolactone 100 mg (B) and of quinethazone 50 mg + furosemide 160 mg + spironolactone 100 mg (C). The results are given in Tables V and VI and in Fig. 3

1 Results of variance analysis. Although the responses to treatment A were lower than those to treatment B, no significant differences were present. However the effects of all drugs combined (C) were significantly larger than those of both other treatments as shown by the comparisons (A−C) (B−C) and (A+B−2C) (Table V and Fig. 3).

2 The combination of all drugs (C) compared

Table VI. *Statistical analysis of the effect addition of all three drugs. Net effect of combination (C (A+B))*

Parameters	Units	Mean value	S.E.	t-value	p-value
Urinary Na	mEq/24 h	+123	±45	2.726	<0.025
Urinary Cl	mEq/24 h	+106	±36	2.925	<0.025
Urinary K	mEq/24 h	30	±16	1.829	>0.10
Urinary Na+K	mEq/24 h	93	±36	2.607	<0.025
Δ Body weight	kg	−1.73	±0.31	5.563	<0.0025

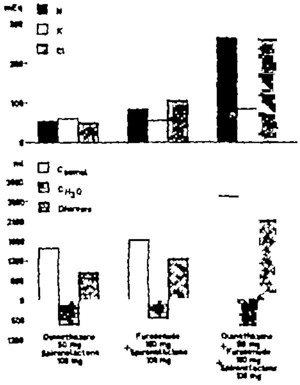


Fig. 3. Mean values for renal electrolyte excretion, osmolal clearance, free water clearance and diuresis in relation to diuretic treatments in trial 3.

to the sum of other treatments $(A+B)$. The differences $(C-(A+B))$ represent the net effects of the combination of all drugs as compared to the *sum of the effects of individual diuretics.* The statistical analysis is shown in Table VI. In terms of sodium, chloride and cation excretion and in terms of weight loss these differences are statistically significant ($p < 0.025$).

These results indicate clearly that the effects of the combination of all drugs represent a supra-additive effect addition or supra-additive summation (7).

DISCUSSION

The present study shows clearly that quinethazone and bendroflumethiazide both present a significant natriuretic and diuretic effect when given as supplementary diuretics in patients receiving long term treatment with furosemide and spironolactone. A definite response was present in 11 out of 12 patients, and the average increase in sodium output amounted to more than 100 mEq sodium/day. It is also evident from this study that the quantitative effects of quinethazone 50 mg and bendroflumethiazide 5 mg are nearly the same. The results indicate that the enhancing natriuretic effect is a common property of the thiazide and phthalimidine compounds, which have a major site of action in the distal renal tubules and which are very similar in terms of saluretic potency and pharmacological action (1, 9, 28).

The second trial was performed in the same clinical setting and demonstrated that the effect of quinethazone 50 mg was definitely superior to that of additional furosemide 80 mg. In a previous study (19) it has been shown that furosemide 80 mg has a significantly larger effect than quinethazone 100 mg when the drugs are used as single diuretics in patients with congestive heart failure. Therefore, the combined effects of the drugs obtained in this study cannot be described as a dose addition effect, but must be explained as an effect addition or a summation produced by diuretics acting by different mechanisms or at different sites in the nephron (7, 18). In accordance with this interpretation it is well established that, in comparison with benzothiadiazine diuretics, furosemide is able to inhibit renal tubular sodium reabsorption more extensively throughout the nephron including the ascending limb of Henle's loop (1–4, 8, 17, 19, 23, 25, 27).

It appears from the third trial that the response to the combination of diuretics represents more



Fig. 4. Schematic diagram of the combined effects of diuretics upon renal tubular sodium transport. A detailed explanation of the mechanism of interaction is given in the text.

than a simple summation of their individual effects. In terms of sodium, chloride and cation excretion and of weight loss the response to quinethazone + furosemide + spironolactone is significantly larger than the sum of the responses to quinethazone + spironolactone and to furosemide + spironolactone. It may be concluded, therefore, that the combined effects of the diuretics represent a supra-additive effect addition or a supra-additive summation (7).

A tentative explanation of the supra-additive effect addition observed is given in Fig. 4. The renal tubular reabsorption of sodium represents the sum of multiple discrete functions throughout the nephron. The major part of sodium is reabsorbed in conjunction with chloride or bicarbonate as represented by the arrows to the left and in the middle of the upper part of the diagram. A minor fraction of sodium is reabsorbed in exchange with potassium in the distal segment of the nephron as shown by the arrow to the right.

With the administration of a saluretic and spironolactone the former drug will depress the reabsorption of sodium in conjunction with chloride at one site in the nephron and, through this effect, accelerate sodium reabsorption at more distal sites by means of the increased supply of sodium and by means of homeostatic mechanisms activated during natriuresis (14, 17), while spironolactone is able to depress the tendency to acceleration of the sodium-potassium exchange. This pattern of action of the drugs is shown in the middle part of Fig. 4.

In this setting it becomes possible for a second saluretic, through its action at the sites of accelerated reabsorption, to promote a more marked natriuresis than it would be able to induce when used alone. Because of the increased delivery of sodium to the sodium-potassium exchange sites the potassium excretion is increased in spite of the administration of spironolactone. This pattern of interaction of the drugs is shown in the lower part of Fig. 4.

The fact that the combined tubular effects of all three drugs involve an increase of the sodium-potassium exchange in the distal tubules appears to suggest that the increased natriuretic effect of the combination of diuretics is more dependent upon the action of the saluretics quinethazone and furosemide, than upon the [illegible] drug spironolactone (1, 2).

The clinical significance of the [illegible] sodium-potassium exchange after the combination of all three drugs is that the increased natriuresis is associated with a marked increment of renal potassium and chloride output. As a result there is a trend to development of hypokalaemia, hypochloraemia and metabolic alkalosis as shown in Table III. This type of electrolyte disturbance carries a risk of development of digitalis toxicity in patients receiving a maintenance dosage of this drug.

A clinical corollary to these findings is, therefore, that supplements of potassium chloride should be considered in order to prevent severe serum electrolyte disturbances when this type of combined diuretic treatment is used.

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Congress Announcement

The 16th Annual Clinical Conference will be held at the Shamrock Hilton Hotel in Houston, Texas, USA, 11–12 Nov 1971 *Topic* Endocrine and non-endocrine hormone producing tumors.

Sponsored by the University of Texas M. D Anderson Hospital and Tumor Institute at Houston and the University's Division of Continuing Education of the Graduate School of Biomedical Sciences.

Seven major sessions will deal with clinical, diagnostic and research aspects of non-endocrine hormone producing tumors, pituitary tumors, pancreatic tumors, primary aldosteronism and other adrenal tumors, parathyroid tumors and the thyroid tumors, hydatidiform and choriocarcinoma.

A symposium on endocrine tumors will be sponsored on Nov 13 by M D Anderson's Department of Anatomical Pathology and the Texas Society of Pathologists. The meeting will be held at Anderson auditorium, Texas Medical Center

Inquiries about the conference Mrs Jane Brandenberger Information Coordinator The University of Texas M. D Anderson Hospital and Tumor Institute Texas Medical Center Houston, Texas 77025 USA

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TRANSIENT CHOLESTASIS DURING TREATMENT WITH AJMALINE, AND CHRONIC XANTHOMATOUS CHOLESTASIS AFTER ADMINISTRATION OF AJMALINE, METHYLTESTOSTERONE AND ETHINYLESTRADIOL

Two Case Reports

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Abstract. Intrahepatic cholestasis and jaundice have been observed in one male and one female patient treated with ajmaline. The male patient improved rapidly upon interruption of therapy. The female who, in addition, had been given methyltestosterone and ethinylestradiol, developed pronounced xanthomatous cholestasis that has persisted for more than two years. There has been no evidence of liver failure in this case. Galactose tolerance tests have been normal. Liver slices from the patient metabolized methyltestosterone in a similar way and to the same extent as those of a control subject. The ultrastructure of many of the liver parenchymal cells appeared normal, whereas others contained vacuoles filled with irregular clumps of electron-dense material—"whorl bodies"—believed to represent altered, and reabsorbed, bile. Similar bodies reflecting regurgitation of bile, probably through diacytosis, were also encountered in the Kupffer cells and in the extracellular spaces (the sinusoids, the bile capillaries and intercellularly between the parenchymal cells). One year after the onset of symptoms there was no evidence of biliary cirrhosis. The observations suggest that the combined effects of several cholestatic agents may result in a more severe cholestatic disease than occurs when either of the drugs is administered alone.

Intrahepatic cholestasis is occasionally observed in patients treated with chlorpromazine, methyltestosterone and other 17α-alkylated steroids (15, 18). This complication has also been reported in two patients (5) for whom ajmaline (an alkaloid isolated from Rauwolfia serpentina) was prescribed. Drug-induced cholestasis usually disappears after interruption of the therapy but chronic liver disease has developed in patients treated with chlorpromazine (13).

The purpose of this report is to describe two patients showing intrahepatic cholestasis following treatment with ajmaline. In addition one of them was given a preparation containing methyltestosterone and ethinylestradiol. This patient developed chronic cholestasis and a clinical syndrome similar to that of xanthomatous biliary cirrhosis.

METHODS

Histological studies

Liver biopsies were performed by the Menghini technique. For light microscopic studies the tissues were fixed in 4% formaldehyde and embedded in paraffin; the sections were stained with haematoxylin and eosin, van Gieson connective tissue stain, and by Perl's method for the demonstration of iron.

The specimens investigated in the electron microscope were fixed in s-collidine-buffered osmium tetroxide, dehydrated in ethanol solutions of increasing strength and propylene oxide, and embedded in Epon. The thin sections were stained with lead citrate and studied and photographed in a Siemens Elmiskop 1 electron microscope.

Metabolism of methyltestosterone

Operative biopsy specimens were used in the studies of the metabolism of methyltestosterone in vitro. The specimens were taken immediately after the laparotomy and transferred into ice-cold saline. The tissues were then cut into thin slices, pieces of which weighing 100–200 mg were incubated in an atmosphere of 95% oxygen and 5% carbon dioxide for one hour at 37°C in 5 ml 0.1 M KH_2PO_4 (pH 7.8) containing 0.5 μCi methyltestosterone 4-C^{14} (11.1 mCi mM, Radiochemical Centre, Amersham, England) and 0–25 μg of unlabelled methyltestosterone. The labelled material was then extracted with chloroform-methanol (1 : 1) at reflux temperature for 30 min. After removing the undissolved residue by filtration and

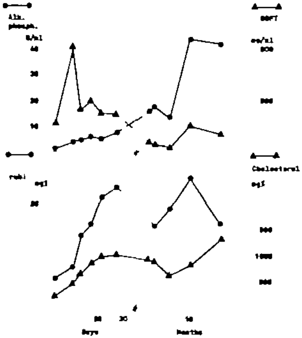


Fig 1 Case 2. Serum levels of bilirubin, alkaline phosphatase, GPT and cholesterol

the organic solvent by evaporation under vacuum, the unconjugated and conjugated steroids were extracted separately with ethyl acetate and ether/isopropanol (3 1 /), respectively (8). The unconjugated steroids were separated by thin-layer chromatography using ethyl acetate/hexane/acetic acid (72 13.5 10 /v) as the solvent system. Areas with labelled spots, detected by autoradiography were scraped off and extracted with chloroform/methanol (1 1 /v). The extracts were transferred into counting vials and assayed for radioactivity after evaporation of the solvents under stream f air

CASE REPORTS

Case 1

A 54-year-old man with history f episodes of palpitations during the past six years. On such an occasion, in Sept. 1969 ECG showed atrial fibrillation and X-ray revealed slight enlargement of the heart. There was no evidence f heart decompensation. On Sept. 27 1969 the patient was prescribed ajmaline (Gilurytmal® 50 mg/tablet), meprobamate (Restenil® 400 mg/tablet) and pentaerithrol (Nitropent, 30 mg/tablet) The tablets were taken sporadically On Oct. 15 the day after the intake of the sixth ajmaline tablet, the patient developed fever (38.0°C) and epigastric distress. During the next few days he noted jaundice, generalized pruritus, dark urine and pale stools. On Oct. 22 his bilirubin value was 4.8 mg/100 ml. All symptoms disappeared within 1–2 weeks. Oral cholecystography during this period was normal.

On Nov 14, 1969 the patient took two tablets of ajmaline. Except for fever the same symptoms recurred within 6 h. O admission to hospital on Nov 16 the liver spleen and lymph nodes were not enlarged, nor were there any clinical signs of liver cirrhosis. Bilirubin in serum was elevated (1.5 mg/100 ml), as were the alkaline phosphatase (4.6, normal range < 2.5 U/ml) and GPT (130, normal range < 40 U/ml). Sedimentation rate, Hb and white blood cell count were normal. Serum γ-globulin was somewhat elevated (1.85 g/100 ml), as was thymol turbidity (6.1–9.5 U/ml, normal range < 4 U/ml). Total serum protein and prothrombin-proconvertin index were normal.

The symptoms disappeared within few days and the laboratory data became normal. An intravenous cholangiography was performed when the patient had almost recovered. It showed no signs of extrahepatic bile duct obstruction. A bromsulphalein retention test was normal 14 days after the last intake of ajmaline.

Case 2

A 52-year-old female typist, who has never been pregnant. In 1932 she was jaundiced for one week. She remembers having recovered rapidly but has no furthe recollections about this episode. A slight ankle edema has been observed intermittently since 1952, but until the beginning of the present illness, in 1968, there had been no evidence of liver kidney or heart disease. A diuretic (3-hydroxi-3-(4-chlor 3-sulfamoyl-phenyl)1-oxoisoindolin) (Hygroto ®) has been prescribed occasionally to relieve the edema. Menstruation was regular until the presence of myoma demanded supravaginal amputation of the uterus in 1967 Since 1963 the patient has had episodes of palpitations, anxiety and flushing of the face. Some of these symptoms were considered due to the menopause, and in May 1968 her doctor prescribed Primodian® 3 tablets daily for 10 days (4 mg methyltestosterone and 2 μg ethinylestradiol/tablet). She responded well to this treatment. In June she started to feel tired and complained of slight headache.

On Aug. 27 1968, the same dose of Primodian® was again prescribed, this time in combination with 150 mg jmaline daily On Sept. 7 the patient noticed pains in several joints, accentuation of the headache and fever (38.0°C) She also observed that her urine was dark On the second day of illness she was admitted to hospital with jaundice and slight fever The liver and spleen were not palpable. There were no edema, characteristic spiders, erythema of the palms, or other clinical signs of hepatic cirrhosis. The levels of bilirubin, alkaline phosphatase and serum cholesterol increased rapidly until the end of the first month of hospitalization (Fig 1). Laparotomy and operative cholangiography ere per formed one month after the onset of jaundice. The gallbladder appeared normal and contained no stones. There was no evidence of extrahepatic bile duct obstruction. Generalized pruritus resistant to treatment with cholestyr amine (16 g/day) ppeared. On reoperation in Feb. 1969 the operative cholangiography was normal. A T-tube as inserted into the common bile duct During the first 4 h the bile portion flowed slowly and was dark green. Subsequent portions ppeared less viscous and ere often brownish-yellow The amount of bile drained varied between 50 and 350 ml/24 h. Following the operation there

was prompt disappearance of the itching, but the serum levels of bilirubin, GOT GPT alkaline phosphatase and cholesterol remained unchanged. Glucocorticosteroids were given on two occasions, before as well as after the application of the bile drainage. No effect of this therapy was observed. Both free and conjugated bile acids were administered to stimulate the flow of bile. Small doses were ineffective; large doses resulted in itching.

Hb, thrombocytes, white cell count, prothrombin proconvertin index, serum iron and transferrin were within the normal range. The ESR varied between 50 and 88 mm/h. Electrophoresis of serum proteins revealed somewhat low albumin fraction and slight increase of the α_2, β- and γ-globulin fractions. In repeated serological tests no serum antibodies against liver, kidney or smooth muscle were demonstrable; neither were any antinuclear factors found. There were no signs of hemolysis. The predominant part of the bilirubin in serum was conjugated. Repeated L-galactose tolerance tests were normal.

Besides the pronounced elevation of serum cholesterol, the patient showed moderate hypertriglyceridemia not exceeding 475 mg/100 ml. Electrophoresis of serum lipoproteins on starch gel revealed small α- and broad β band.

The patient has now been under observation for almost 2 years. During the last year she developed marked xanthelasma and xanthoma on her hands, elbows and buttocks. She has been tired but otherwise in relatively good condition. For periods of 1 3 weeks she has been able to work halftime. The T-tube was removed after 8 months without recurrence of the intensive pruritus. The liver and spleen have not become enlarged. Hepatic vein catheterization was performed when she had been icteric for 1 / years. The occluded as well as the free hepatic venous pressure were normal.

MORPHOLOGY OF LIVER BIOPSIES

Case 1

A liver biopsy was performed when most symptoms had disappeared. It showed slight dilation of some bile capillaries, some of which contained biliary thrombi. Otherwise there were no pathological changes in the tissue.

Case 2

Biopsies were performed on 5 occasions, from Sept. 1968 to the end of Aug. 1969. The first 4 specimens (2 operative, 2 Menghini) were studied in the light microscope, while the one obtained in Aug. 1969 (Menghini) was also investigated in the electron microscope.

Liver specimens obtained in Sept. and Oct. 1968 showed severe cholestasis, numerous bile thrombi in the bile capillaries, and bile pigments in the cytoplasm of hepatic parenchymal cells. Inflammatory changes were either minimal or absent, the portal areas were unremarkable, and there were no signs of degeneration or necrosis of the hepatocytes or other types of cells.

I Jan. and March 1969 when the patient had been icteric for 4 and 6 months, respectively there were still signs of severe cholestasis. I addition, small, randomly scattered areas with degenerative cellular changes and frank necrosis were observed. These areas were surrounded by focal collections of inflammatory cells, mainly polymorphonuclear leukocytes.

Light microscopy of the biopsy specimen obtained in Aug. 1969 revealed numerous droplets of bile pigment in the hepatocytes. Similar droplets f yellowish-green pigment were observed in some of the cells lining the sinusoids. Inflammatory alterations were notably absent, but focal areas with the "feathery" "reticular" or vacuolar type of degeneration were observed. Although there was slight diffuse increase in the collagenous reticulum surrounding individual parenchymal cells in some lobules, cirrhosis of biliary or other type was not demonstrated.

Electron microscopy of this specimen revealed completely normal structure in many of the parenchymal cells. Sections of other cells showed the presence of vacuoles ranging in size from 0.2 to 8 μ and containing irregular clumps of dense and granular material (Figs. 2 and 3). Further analysis revealed that the clumps of dense material often had substructure, with the appearance of irregular membrane whorls often surrounded by strands of membranous or flocculent material (Fig. 3). Similar whorl bodies were frequently encountered in the extracellular—often wide—space between lateral cell membranes of adjacent cells, and in the sinusoids (Fig. 3). Although occasional bile capillaries were of normal appearance with regularly shaped, slender microvilli (Fig. 4), the majority of the capillaries showed either one of the following alterations or combination of them: dilatation (Fig. 2), reduction in number and height of microvilli (Fig. 2) occurrence of whorl bodies (Fig. 4), or presence of finely granular or flocculent material.

Most of the Kupffer cells were enlarged and contained abundant single-membrane limited cytoplasmic bodies of highly variable size, shape and appearance (Figs. 5 and 7). The majority of these bodies had moderately electron-dense background "matrix" containing small irregular clumps of dense, ill-defined material. Other cytoplasmic bodies appeared as vacuoles with "whorl bodies" or strands of membranes suspended in clear "watery" matrix. In addition, the cytoplasmic ground substance of the Kupffer cells contained rounded electron-lucent areas with fine structure suggestive of neutral lipid ("lipid droplets"). Infrequently "cytosegresomes" (7) composed of more or less altered cytoplasmic organelles were noted (Fig. 6). Microvillous proliferations of the sinusoidal surfaces of the Kupffer cells were often observed (Fig. 7). In some areas slight increase in the collagen located in the extracellular space was noted (Fig. 3).

In none of the biopsy specimens was there any evidence of bile duct proliferation, cholangitis, cirrhosis, or hepatitis, nor did the morphological observations reveal pathologically increased numbers of lipid droplets in the parenchymal cells.

METABOLISM OF METHYLTESTOSTERONE IN VITRO

Operative biopsy specimens of the liver from case 2 (second operation) and female control subject (aged 53



Fig. 2. Portions of hepatic parenchymal cells containing vacuoles (V_1 and V_2) with granular (arrows in V_1) and dense membranous material. The latter material often consists of concentric whorls (forming "whorl bodies"). *L*, bodies with the appearance of conventional lysosomes. *Inset* shows dilated bile capillary (*BC*) containing finely granular material. *D*, desmosome. ×21 000; *inset* ×12 500.



Fig. 3. Portions of two adjacent parenchymal cells (PC_1 and PC_2) and Kupffer cell (*KC*). PC_1 contains several vacuoles with "whorl bodies" (*WB*). The extracellular space (*ES*) is widened and contains abundant "whorl bodies". Two of these are boxed and are shown at higher magnification in the insert. The concentric arrangement of the membranes in these bodies is brought out in the latter. Note presence of whorl body (*arrow*) in the sinusoidal space (*S*). *Col* collagen. 15 000, insert 40 000.



Fig. 4 Variable appearance of three bile capillaries (*BC*). The one in the upper portion is greatly dilated and can be identified by the presence of small remnants of microvilli (*Mv*). "Whorl bodies" are present both in this dilated capillary and in the one located immediately below. A third bile capillary shows normal structure. *WB* "whorl body" in a cytoplasmic vacuole of a parenchymal cell. ×20 000.

cholecystectomy due to cholelithiasis) were incubated with methyltestosterone-4-C^{14}.

The proportion of radioactivity recovered as "unconjugated" and "conjugated" steroids was essentially the same for both patients irrespective of whether unlabelled methyltestosterone had been added to the incubation mixtures or not. In the presence of 25 µg of the unlabelled steroid, 5% of the radioactivity was found in the ether-isopropanol fraction from both subjects. Autoradiograms of the thin-layer chromatograms of the unconjugated steroids showed a major spot with an Rf value identical to that of methyltestosterone. It contained 75 and 86% of the radioactivity recovered from the plate in the patient and in the control subject, respectively. The remaining radioactivity was distributed among three other spots. The pattern of distribution was almost equal in the two subjects. No attempt to identify the metabolites was made.

DISCUSSION

The unexpected development of jaundice in these two patients within two to three weeks after the start of therapy indicates that the drugs contri-

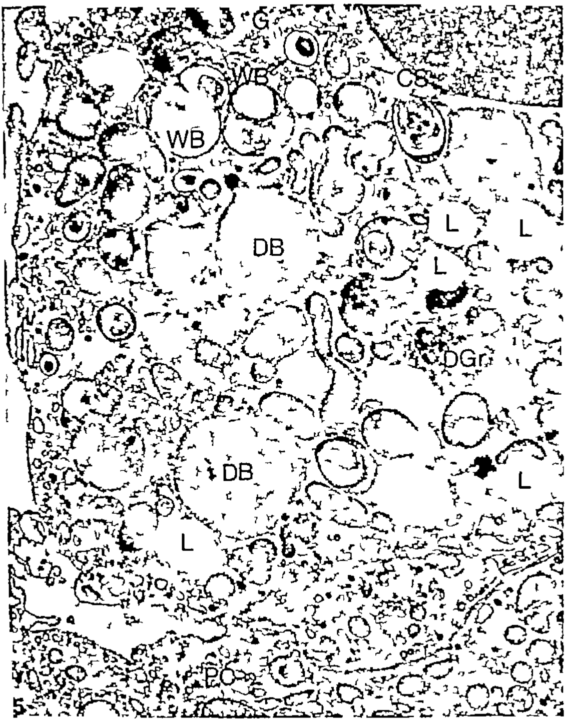


Fig. 5. An enlarged Kupffer cell (*upper*) contains abundant inclusions of variable appearance, such as dense bodies (*DB*) characterized by homogeneously dense matrix, bodies with dense and granular content (*DG*); vacuoles with whorl bodies (*WB*); cytosegresome (?) (*CS*) and lipid droplets (*L*). *PC* sinusoidal area of parenchymal cell; *G* Golgi region. ×16 000.

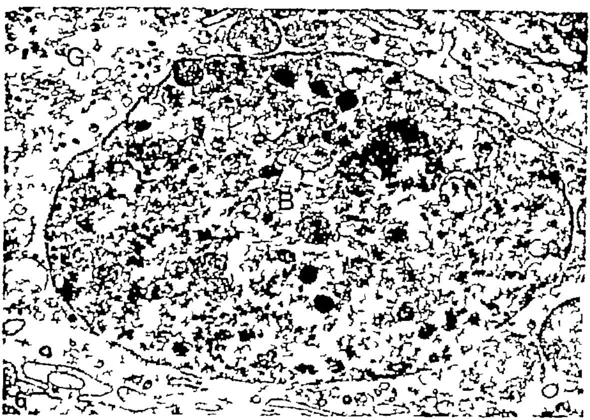




Fig 6 Portion of Kupffer cell containing giant body (*B*) with the appearance of cytosegresome This body may either signify heterophagocytosis or autophagy (?). *G* Golgi apparatus. 21 000.

Fig 7 Part of Kupffer cell containing numerous dense bodies (*DB*) vacuole (*V*) with dense bodies and concentrically arranged lamellar material; and small bodies with granular content. Note extensive microvillous proliferation (*mv*) of the surface of the cell. 18 000

buted to the hepatic injury. Patient 1 received three drugs: ajmaline, meprobamate and pentanitrol. The course of his disease did not differ from those previously reported for two German patients who developed jaundice during treatment with ajmaline (5). The jaundice was mild and the symptoms disappeared shortly after withdrawal of the drugs. Subsequent liver function tests gave no evidence of residual disease.

Patient 2 was prescribed ajmaline, methyltestosterone and ethinylestradiol. The development of symptoms was more dramatic than in the first case and they did not disappear upon interruption of administration of the drug.

In both cases the clinical diagnosis of cholestatic icterus was supported by the morphological picture of the liver biopsy specimens. Patient 1 showed a few bile thrombi and dilatation of the bile capillaries, i.e., changes similar to those previously described after administration of ajmaline (5), 17α-alkylated steroids and phenylbutazone (11 15 16).

Although severe, the structural changes in the liver of patient 2 were in part similar to those previously observed in extra- and intrahepatic cholestasis in man (1 4 20). Inflammatory changes were either slight or negligible and there was no evidence of cirrhosis even in the latest biopsy specimen, obtained 12 months after the onset of symptoms.

Previous studies of cholestasis in man and experimental animals have shown that retained bile material may exist in at least five different forms in the canaliculi: (*a*) a homogeneous, moderately electron-dense material (*b*) a coarsely granular amorphous material [illegible] a rather marked electron density ( ) a precipitate with a crystalline structure forming rosettes of needles, (*d*) an aggregate of lipids (lipid emboli) and ( ) spherical or more irregularly shaped "lamellar material (1 4 9 20). The histological liver structure encountered in patient 2 was not entirely compatible with any of these forms, although it showed some resemblance to the lamellar material described by Hübner (9). The retained material appeared as whorl bodies composed of concentric lamellae of membranous material along with some dense and granular substances. The whorl bodies were localized not only in the bile capillaries but also in the vacuoles of many hepatocytes, both in the sinusoids and intercellularly.

Recently Matter et al. (1) were able to demonstrate that horseradish peroxidase (a protein identifiable at the electron microscopic level) was rapidly transported to the sinusoids when injected retrogradely into the common duct of rats. It appeared that peroxidase was taken up by the hepatocytes from the lumen of the bile capillaries by way of endocytosis. The endocytotic vacuoles seemed to stream to the sinusoidal surface of the cells and to empty their content into the sinusoidal space through exocytosis. Thus material from the bile capillaries may regurgitate to the sinusoids by bypassing, and not necessarily by involving damage or rupture, of the tight junctions at the capillary side of the cell ("diacytosis").

These observations (12) may offer an explanation of the phenomenon observed in patient 2. The presence of "whorl body -containing vacuoles in many hepatocytes should then indicate that the material in the vacuoles has been taken up by the cells from the inspissated bile by endocytosis (t the capillary surface). The extracellular whorl bodies and those found in the sinusoids and in Disse's space should represent material that has reached these locations by a diacytotic process. If so, the presence of "whorl body"-containing vacuoles in the Kupffer cells is simply explained by the assumption that the bodies had been endocytized once again, this time by the Kupffer cells. The cytoplasmic inclusions in these cells probably also reflect other processes such as an uptake of lipids (occurrence of lipid droplets) and damaged cells or cell particles (occurrence of cytosegresomes") (7).

The mechanisms that contribute to development of intrahepatic cholestasis are unknown (2, 10 17). Studies in vitro in case 2 failed to provide evidence for an abnormal liver metabolism of methyltestosterone. A hydrostatic dilatation of the bile capillaries appears to be an early phenomenon (10, 19). Altered permeability or cellular breakdown may result in an excretion of protein that may produce precipitation of the materials on the bile (6). The presence of whorl bodies in patient 2 can possibly be explained by the assumption that the chronic cholestasis has resulted in structural reorganization of inspissated bile. However it may with equal ambiguity be proposed that the unusual structural form of the bile reflects specific change of the bile itself. The appearance of the bile portion collected through

the bile drainage during the first 24 hours may be taken to support this latter assumption. Although the gallbladder was empty the bile specimen was dark green and apparently very viscous. An increased viscosity and a reduction of the solutes of the bile may be important factors in the pathogenesis of the plugging of the bile capillaries.

Patient _ showed no signs of hepatocellular failure the galactose test was normal. In accordance with these findings there was no evidence of subcellular damage in the hepatocytes outside *areas with the typical feathery" type of degeneration.*

Although there are numerous reports suggesting that administration of 17 -alkylated steroids may result in cholestasis, there is no indubitable evidence of the development of chronic jaundice (18). It is possible that the combined effects of several cholestatic agents may result in a more severe cholestatic disease than occurs when either of the drugs is administered alone.

Note added in proof

Since the preparation of this paper the xanthomatosis of case 2 has increased gradually The laboratory data have remained essentially constant. The patient is suffering badly from pains in her hands and feet An operative liver biopsy specimen obtained _ years after onset of symptoms showed chronic cholangitis and biliary cirrhosis. The mitochondrial immunofluorescence test was still negative in Oct. 1970

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THE CARDIAC RESPONSE TO EXERCISE IN AORTIC VALVULAR DISEASE BEFORE AND AFTER BALL VALVE REPLACEMENT

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Abstract. Cardiac output, stroke volume, "exercise factor" and maximal arterial oxygen transport during upright exercise have been determined in seven patients with aortic valvular disease before and after successful ball valve replacement, in four patients with persistent heart failure after ball valve replacement and in four healthy subjects. Preoperatively the increment in stroke volume during exercise was subnormal, but the "exercise factor" was often within normal limits. Patients with heart failure showed definitely reduced "exercise factor" and maximal stroke volume. After successful ball valve replacement both the maximal stroke volume and "exercise factor" increased slightly but insignificantly and with considerably scattered individual values. However all patients with postoperatively increased "exercise factor" had also acquired a fall in Hb concentration. Thus the maximal oxygen transport capacity during submaximal exercise was not improved by the operation.

The hemodynamic consequences of aortic valvular disease are well known (2, 3, 8, 9, 14). The hemodynamic result of an aortic valve replacement is less well documented (2, 12) several of the studies have been made only during rest. Clinical and hemodynamic improvement following heart valve replacement is reported in most patients (4, 5, 11), though some patients deteriorate or fail to improve. The objective distinction between a good and poor result has in many cases been difficult (3, 14, 15).

In the present study we have examined the cardiac response to exercise in aortic valvular disease before and after ball valve replacement with the dual purpose to assess the cardiac function in unoperated aortic valvular disease and to evaluate the effect of ball valve replacement.

MATERIAL AND METHODS

Three groups of subjects were investigated. Group I consisted of seven patients with unoperated aortic valvular disease, two with dominant regurgitation and grossly enlarged hearts and four with rather pure valvular stenosis. The patients of group I were studied before and 4 to 10 months after replacement of the aortic valve with Starr-Edwards prosthesis (Table I). All patients in this group had sinus rhythm both pre- and postoperatively. Slight reduction of the heart size was demonstrated postoperatively and the ECGs suggested lessened left ventricular overload (Table I).

Group II included four patients with poor clinical results after insertion of aortic ball valves, with grossly enlarged hearts, arrhythmias and chronic heart failure classified as functional class 3 and 4 (N.Y.H.A.) (Table IV).

Group III consisted of four healthy subjects. The two latter groups were included in the study in order to outline the quantitative differences between normal and poor cardiac function with the method applied.

Diagnostic preoperative investigations included retrograde catheterization of the left ventricle with cineangiographic study of the aortic and mitral valves and the coronary arteries. Cardiac output was determined in supine and sitting position at rest, and during upright exercise on a mechanically braked bicycle ergometer. Cardiac output was measured by the indicator technique, using indocyanine green, and calculated from the records obtained with the Beckman cardiodensitometer. The work load was increased stepwise from 300 to 750 kpm/min, aiming at a final submaximal load which provoked a heart rate of 120 to 150. Cardiac output was determined during steady state as judged by a constant heart rate usually between the 9th and the 13th min. Duplicate estimations were made both at rest and during exercise. The postoperative investigation followed the same schedule. When possible, the patients performed exactly the same work as preoperatively. Angiographic studies and measurement of pressure gradient were not made postoperatively.

In order to correct for individual differences in work load a so-called "exercise factor" was calculated from the values obtained with the patient in sitting position.

$$\frac{CO_{ex} - CO_{rest}\ (\text{ml/min})}{\text{work load (kpm/min)}}$$

Table I. *Pre- and postoperative radiographic heart size and ECG pattern in group I*

AS = aortic stenosis, AI = aortic incompetence, LVH = left ventricular hypertrophy, RBBB = right bundle branch block, S–E = Starr–Edwards

	Preoperative						Postoperative				
Pat. no.	Age (y)	Sex	Diagn.	Heart vol. (ml/m²)	ECG diagn.	SV + RV (mm)	Prosthese	Interval (mo.)	Heart vol. (ml/m²)	ECG diagn.	SV + RV (mm)
1	61	♀	AS AI	455	LVH	40	S–E 8	10	430	RBBB	—
2	44	♂	AI	680	LVH	52	S–E 14	5	540	LVH	44
3	33	♀	AS AI	530	LVH	52	S–E 10	7	435	LVH	43
4	51	♂	AS AI	500	RBBB	—	S–E 10	6	510	RBBB	—
5	58	♂	AS	485	LVH	55	S–E 9	6	370	LVH	35
6	36	♂	AS AI	535	LVH	40	S–E 11	8	535	LVH	40
7	52	♂	AS AI	765	LVH	35	S–E 13	4	770	LVH	27
Mean	48			564		45			512		37

where $CO_{ex} - CO_{rest}$ is the increment in cardiac output during steady state exercise in the sitting position. In order to correct for changes in the oxygen binding capacity of the blood, the maximal oxygen transport capacity was calculated and related to the work load.

$$\frac{\text{maximal } O_2 \text{ transport (ml/min)}}{\text{work load (kpm/min)}}$$

For statistical evaluations of the pre- and postoperative data the *t*-test was applied.

RESULTS

In group I both supine and sitting mean resting cardiac output was unchanged after the operation (Table II). Subnormal resting cardiac output was found in cases 5 and 7 preoperatively and in cases 2 and 7 postoperatively. Two patients were not able to perform the same work after operation as before, but nevertheless the mean maximal cardiac output during exercise showed a slight increase. The ability to increase cardiac output during work, expressed as exercise factor, was not significantly increased ($p = 0.10$). Preoperatively the stroke volume increased during exercise in all but two patients, the mean increase being 27%. Postoperatively all patients were able to increase their stroke volume, on average by 39%. The maximal stroke volume after operation was not significantly higher than before (8%) nor

Table II. *Clinical diagnosis and pre- and postoperative hemodynamic findings in group I*

AS = aortic stenosis, AI = aortic incompetence, CO = cardiac output (sitting), SV = stroke volume, HR = heart rate

	Preoperative							Postoperative					
Pat. no.	Diagn.	Press grad. (mmHg)	CO (l/min)	SV	HR	Work load (kpm/min)	Exercise factor		CO (l/min)	SV	HR	Work load (kpm/min)	Exercise factor
1	AS AI	90	4.1	48	85			Rest	3.8	42	85		
			7.0	46	150	300	9.6	Ex.	8.8	58	150	300	16.6
2	AI	—	7.0	77	90			Rest	3.7	51	73		
			13.5	91	153	600	10.8	Ex.	9.6	80	120	600	9.8
3	AS AI	20	3.9	52	75			Rest	3.8	45	83		
			7.6	53	142	300	12.3	Ex.	7.7	51	148	300	13.0
4	AS AI	108	3.4	42	80			Rest	5.3	57	92		
			8.0	71	112	450	10.0	Ex.	10.2	91	112	300	16.3
5	AS	100	4.1	49	83			Rest	5.1	66	77		
			8.8	70	125	450	10.4	Ex.	10.8	74	145	450	12.6
6	AS AI	78	4.2	60	71			Rest	5.3	75	70		
			10.3	80	126	600	10.1	Ex.	12.4	95	130	600	11.8
7	AS AI	40	3.6	60	60			Rest	3.6	60	57		
			12.1	80	150	750	11.3	Ex.	9.6	84	140	600	10.0
Mean			4.3	55	77	492		Rest	4.4	56	76		
			9.6	70	137		10.6	Ex.	9.9	76	135	450	12.9

Table III. *Hb concentration and calculated maximal arterial oxygen transport during exercise related to work load (group I)*

	Preoperative		Postoperative	
Pat. no.	Hb (g/100 ml)	ml O_2/kpm	Hb (g/100 ml)	ml O_2/kpm
1	14.0	4.4	11.8	4.4
2	16.0	5.0	15.4	3.3
3	14.5	4.9	13.6	4.7
4	12.2	3.0	9.3	4.2
5	15.7	4.1	13.3	4.2
6	15.4	3.2	13.9	3.8
7	15.7	3.3	14.8	3.1
Mean	14.7	3.98	13.1	3.95

was the ability to increase the stroke volume significantly improved. At a certain work load the heart rate increased somewhat more after the operation.

Following the operation the mean Hb concentration of group I fell from 14.7 to 13.1 g/100 ml ($p < 0.005$) (Table III). When the Hb concentration and the maximal cardiac output were combined in the entity maximal arterial oxygen transport per unit work load, no postoperative change at all was found (Table III). In two patients (cases 6 and 7) with preoperative major valvular incompetence the largest prostheses 13 and 14 were inserted. In the postoperative study these two patients were the only ones with postoperative Hb concentration of more than 14.8 g/100 ml, and an exercise factor which was lower than during the preoperative study.

All patients in group II were able to perform exercise of 450 kpm/min (Table IV). The heart rate response and resting cardiac output were within the limits of group I. There was, however, a mean decrease in stroke volume during exercise of 16% and in all patients the "exercise factor" was lower than in group I.

The cardiac response to exercise in the healthy subjects of group III was similar to that of group I. Stroke volume increased during exercise by 36% on average (Figs. 1 and 2).

DISCUSSION

Procedures for testing the maximal working capacity in healthy subjects are not always valid in patients with heart disease (6, 16). It is usual to relate cardiac output to the oxygen consumption during relatively light exercise (6). The basis for this approach is the observation that a diseased heart has a reduced ability to respond also to a submaximal load. In the present study the increment in cardiac output following exercise was related to the work load in kpm/min. This approach is probably valid because a linear relationship exists between oxygen consumption and work load during steady state exercise (1, 16).

The present study confirms that cardiac output and stroke volume during rest are often within normal limits in patients with severe aortic valvular disease (3, 5, 12, 14), thus being rather insensitive indicators of cardiac function. During exercise maximal stroke volume is usually attained at heart rates of 110 beats per minute or more in healthy subjects. The normal maximal

Table IV. *Cardiac output during upright exercise in patients in poor clinical condition after aortic ball valve replacement (group II)*

Abbreviations as in Tables I and II

Pat. no.	Sex	Age (y)	Postop. heart vol (ml/m²)	Arrhythmia	ECG	Combined valvular lesion		CO (l/min)	SV (ml)	HR	CO/kpm	Work load (kpm/min)
8	♂	39	625	—	LVH	MS	Rest	4.8	80	62	6.8	450
							Ex.	7.9	53	150		
9	♂	51	920	AF	LVH	MI	Rest	3.5	50	70	8.4	450
							Ex.	7.3	60	120		
10	♂	63	900	—	LBBB	–	Rest	3.8	60	64	8.2	450
							Ex.	7.5	54	140		
11	♂	56	730	AF	LVH	—	Rest	3.9	56	70	8.0	450
							Ex.	7.5	53	140		
	Mean	51	779				Rest	4.0	62	66	7.7	450
							Ex.	7.5	52	138		

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CLINICAL EXPERIENCE WITH A SWEDISH FACTOR IX CONCENTRATE

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Abstract. A factor IX concentrate, prepared by modifications of the method of Tullis and with the supernatant of fraction I-0 as base material, has become available in Sweden. The factor IX activity of the concentrate is about 20 times that of an equivalent volume of normal plasma. The factor IX concentrate, altogether 226 bottles, has been given to 18 patients with haemophilia B in connection with major and minor surgical procedures (7 patients) and bleeding episodes such as joint bleeding, renal bleeding and large haematomas. After injection abnormal bleeding was arrested and the surgical procedures could be performed without any haemorrhagic complications. The factor IX activity in vivo ranged from 16 to 69% (average 38%) of the in vitro activity of the concentrates administered. The half-life of the injected factor IX was about 20 hours. A dose of 10–16 units per kg b.wt. is recommended for raising the factor IX level to 16–20% and a dose of 30–40 units per kg b.wt. for raising it to at least 40%. Four patients developed hepatitis, but otherwise no side-reactions were seen.

Concentrates of factor VIII for the treatment of haemophilia A have been used in Sweden since 1956. But for haemophilia B we have had recourse only to blood and plasma transfusions. Recently however concentrates of factor IX have become available for clinical use.

Soulier et al. (9, 19, 20) have prepared a concentrate of factors II, VII, X and IX. They called the preparation fraction PPSB. The activity of factors II, VII, X and IX per millilitre of the concentrate is 20–30 times higher than that in normal plasma. Fraction PPSB is prepared from fresh human EDTA plasma. The clotting components are adsorbed on tricalcium phosphate and afterwards eluted with citrate and precipitated with 25% ethanol at −5 C. The fraction has been given to several patients with haemophilia B in connection with bleeding episodes and surgical procedures. It has proved to have a good therapeutic effect and no side-reactions have been reported (1, 10, 20). Bidwell et al. (2, 3, 4) have prepared a factor IX concentrate from the residue obtained in the ether fractionation procedure. It contains factor IX in a concentration 6–15 times that in normal plasma.

Tullis et al. (22) have devised a method for preparing factor IX concentrate from plasma by chromatographic adsorption and elution from DEAE cellulose. The activity of this preparation per millilitre is said to be 10–70 times that of factor IX in normal plasma (21, 22). Breen and Tullis (5) reported their experience with this preparation on 14 patients, including 5 with haemophilia B. A concentrate of factor IX, called Konyne® has now been placed on the market by Cutter Laboratories (Berkely, California). The preparation is manufactured by a modification of the method of Tullis. The effluent of fraction I is adsorbed on DEAE Sephadex and eluted fractionally. It contains 10–60 units factor IX per millilitre. Hoag et al. (8) reported a good therapeutic effect of this preparation in 9 patients with haemophilia B.

Recently concentrates of factor IX have been prepared by several workers using modifications of the methods mentioned above. Brummelhuis et al. (6) in Holland have used ACD plasma and aluminium hydroxide for small-scale production of factor IX concentrate of low potency, which has been successfully used in a few patients with haemophilia B. Shanbrom (18) starts with fraction III paste and then uses modifications of the methods of Bidwell et al. (2) and of Soulier et al. (19). Gilchrist et al. (7) have tried this fraction in four patients with haemophilia B with a good therapeutic effect. Pert et al. (15) also start with fraction III and then use DEAE cellulose and tricalcium phosphate.

Table I. *Preinfusion factor IX levels, the highest postinfusion levels attained, number of doses of factor IX administered in connection with surgical procedures*

Case no.	Initials	Fam. no.	Age (y)	Weight (kg)	Type of haemophilia	Surgical procedure	Factor IX level: Before inf. (%)	Factor IX level: Highest after inf. (%)	Doses[a] of factor IX adm.
1	B. Å.	183	22	55	Severe	Achillotenotomy	<1	70	28
2	U. O.	116	14	40	Severe	Achillotenotomy	<1	78	15
3	L. F.	51	16	35	Severe	Incision and revision of giant haematoma with necrosis of right leg and plaster extension of both knees	<1	50	31
4	L. B.	173	16		Moderate	Abortion (abd. hyst.)	2	69	19
5	K. D.	14	8	23	Moderate	Extraction of 2 teeth	3	50	11
6	A. H.	177	32	73	Mild	Extraction of 8 teeth	10	33	8
7	G. G.	99	28	55	Severe + inhibitor	Extraction of 4 teeth	<1	23	14

[a] 1 dose = 1 bottle of factor IX.

Except for fraction PPSB the clinical experience with the various factor IX concentrates is limited.

A factor IX concentrate has recently become available in Sweden. It is prepared by modifications of the method of Tullis and with the supernatant of fraction I-0 as a base material. The present report concerns our experience with the factor IX concentrate in 18 patients with haemophilia B.

MATERIAL AND METHODS

Clinical material. The clinical material consisted of 18 patients with haemophilia B, which was severe in 14, moderate in 3 and mild in 1. Family history, symptoms and coagulation status of these patients have been described elsewhere (1, 14, 16) with the exception of 1 patient, namely Z. G., fam. 265. The patients are referred to by their initials, years of birth, and their family numbers given in the earlier papers.

Z. G., fam. 265 was a 23-year-old man (born 1947) with severe haemophilia B. Ever since his first year of life he had repeatedly had large intramuscular haematomas and joint bleeding, which had caused severe reduction of the mobility of the knees and ankles. He had also had repeated episodes of renal bleeding and intraabdominal bleeding. He had been hospitalized several times and given altogether about 100 blood and plasma transfusions.

Factor IX concentrate. The concentrate was prepared by Kabi, Stockholm, according to slight modifications of the method of Tullis (8, 22). The effluent of fraction I-0 was adsorbed on DEAE Sephadex A-50, eluted fractionally, desalted and lyophilized. The final product was dissolved in isotonic citrated saline, sterilized by filtration, placed in final bottles and lyophilized. Each bottle of freeze-dried fraction contains about 600 mg of protein. The factor IX activity of one bottle (25 ml) corresponds to that of 420–680 ml of fresh human plasma.

Coagulation tests. All methods used for collection and preparation of blood samples as well as for determination of various coagulation factors have been described elsewhere (12). After withdrawal of the blood the plasma was immediately frozen at −60°C.

The factor IX activity of the patient's plasma as well as of the factor IX concentrates was assessed by its normalizing effect on the recalcification time of platelet-rich haemophilia B plasma containing less than 1% factor IX of normal (13). The amount of factor IX was expressed relative to that found for a normal standard consisting of pooled plasma from 10–20 healthy individuals. Each sample was assayed at least twice.

Quantitative determination of inhibitor of factor IX. The plasma to be tested (0.4 ml) was incubated in various dilutions (dil. 1/1, 1/2, 1:5 and 1:10) with concentrate of factor IX diluted 1:5 (0.2 ml) at 37°C for hours. As blank 0.6 ml saline was incubated with 0.. ml of the factor IX concentrate. Following incubation the blank and the mixtures of plasma and factor IX concentrate were assayed for residual factor IX activity in the way described above. The inhibitor activity of the plasma was expressed as the number of units of factor IX (one unit of factor IX is defined as the amount of factor IX present in 1 ml of normal plasma) inactivated by 1 ml of the plasma.

Solution and administration of the factor IX concentrate. An isotonic solution was prepared by dissolving the freeze-dried protein of one bottle at room temperature in 25 ml of distilled water. It was infused in the course of 10–1 min. Usually 3 bottles of factor IX concentrate were given on each occasion.

Blood samples for assay of factor IX were obtained immediately before each infusion, 10–20 min after com-

pletion of the infusion separate venipuncture was, if possible, made for determination of the factor IX concentration attained. The recovery was calculated by relating the value found in vivo to that predicted on the assumption of homogeneous distribution over the intravascular plasma volume of the amount of factor IX concentrate administered.

RESULTS

The Swedish factor IX concentrate, altogether 226 bottles, was given to 18 patients with haemophilia B (Tables I and II). No untoward reactions to the infusions were observed. Three patients had previously developed immune antibodies to blood group antigens, but no reactions to the concentrate were noted. Four patients have developed hepatitis, namely B. G fam. 14 G G fam. 99 L. P fam. 31 and R. B. fam. 59 These patients developed hepatitis 3–4 months after the infusions. With the exception of L. P fam. 31 these patients had received only factor IX concentrates. In all cases the hepatitis was fairly mild. Three patients (J H. fam. 110, S. J fam. 112, G G fam. 99) had inhibitors against factor IX. The inhibitory activity decreased after infusion of factor IX concentrate but increased markedly after 4–6 days. After 1 month the inhibitory activity had again reached the preinfusion level. No circulating anticoagulant in excess was demonstrable in the blood in any of the other patients. No resistance to treatment was demonstrated in patients who received repeated doses of the concentrate in the course of 18 months.

The average in vitro activity of P & P factor VII and factor IX in 18 batches of the concentrate are given in Table III. The mean protein concentration was 24 mg/ml It is apparent that the factor IX activity was about 20 times that of an equivalent volume of normal plasma. Assuming that normal whole plasma contains 70 mg of protein per millilitre, this indicates purification of about 70 times for factor IX.

After 64 single injections of concentrate in altogether 1 patients we calculated the in vivo yield of factor IX. In these patients samples for factor IX assay were obtained immediately before, and again 15 min after the end of the injection. Based on a mean plasma volume of 41 mg/kg b.wt. the factor IX activity in vivo ranged from 16 to 69% (verage 38%) of the in vitro activity of the preparation administered. I patients from whom samples were obtained at close intervals the half-life of the injected factor IX was about 20 hours.

The patients were treated in connection with major and minor surgical procedures and bleeding episodes, such as joint bleeding, renal bleeding and large haematomas. Case reports of the patients subjected to major or minor surgical procedures are given below The number of doses of factor IX concentrate administered in connection with the various surgical procedures are given in Table I.

Table II summarizes the preinfusion factor IX levels, the highest postinfusion levels attained, the dose of factor IX infused and the clinical response in the patients treated in connection with various bleeding episodes. Except for two patients with inhibitors, the factor IX level always rose. All the patients showed clear clinical improvement after the infusion. It is difficult to evaluate the effect of the therapy on haemarthrosis. It was remarkable, however that all patients who had received factor IX reported prompt relief of the pain in the respective joints. In addition, the swelling of the joints usually disappeared within –4 days. No new joint deformities developed in association with joint bleeding treated with factor IX. Even in the two patients with inhibitors the factor IX treatment appeared to have some clinical effect.

CASE REPORTS

Case 1

B A., male, born 1947 severe haemophilia B (fam. 183) Since the age of 5 years he had had recurrent haemorrhage in various joints, mostly the knee and elbow joints. In 1961 he had been admitted to his local hospital because of heavy bleeding into the muscles of the left calf and left knee joint. Since then the ankle had been stiff in almost full extension, and the knee had an extension defect of 20°

I April 1964 the contracture of the knee joint was treated ith *traction and active physiotherapy* During the treatment 400 ml stored plasma as given once or twice eek. After 3 weeks treatment the extension as no longer defective. The ankle joint was unchanged. No bleeding occurred during the treatment.

In Jan. 1965 *achillotenotomy and posterior capsulotomy* was performed There as no abnormal loss of blood during the operation, which as done ithout tourniquet. The ankle could be flexed to 80° position. Plaster as applied below the knee joint and slit up in front. After 2 eeks the plaster and the sutures were removed, after which new plaster as applied for further 2 eeks.

Table II. *Preinfusion factor IX levels, the highest postinfusion levels attained dose of factor IX infused and clinical response in patients treated in connection with various bleeding episodes*

Initials	Fam. no	Age (y)	Weight (kg)	Type of haemophilia	Type of bleeding	No. of episodes	Factor IX level: Before inf (%)	Factor IX level: Highest after inf (%)	Doses of factor IX adm.
L. P	31	16	35	Severe	Haemarthrosis	8	<1	8–18	9
					Bleeding from ruptured ear drum	1	<1	11	1
B. G	14	47	70	Moderate	Ileopsoashaematoma + a cyst in Ileum	11	3	8–17	13
B. Å	183	22	55	Severe	Haemarthrosis	9	<1	8–26	17
S. L.	61	30	65	Severe	Haemarthrosis	1	<1	7	1
K. Pe	31	38	50	Severe	Haemarthrosis	1			1
R. B.	59	16	30	Severe	Aspiration of joint for haemorrh.	1	<1	20	2
					Haemarthrosis	4	<1	8–20	5
K. P	118	14	40	Severe	Haemarthrosis	1	<1	23	3
C S.	241	5	18	Severe	Haemarthrosis	17	<1	13–40	17
S J	112	27	46	Severe	Haematuria	1	<1	5	4
				inhibitor of factor IX	Haemarthrosis	1	<1	5	4
J H	110	18	55	Severe + inhibitor of factor IX	Haemarthrosis and large haematoma	4	<1	<1	11
J Ö	44	15	55	Severe	Haemarthrosis	1	<1	7	1
Z. G	265	23	60	Severe	Haemarthrosis	8	<1	8–16	9
S G	14	42	73	Moderate	Incision of giant haematoma from cyst in ileum	1	4	32	6
K. D.	14	8	23	Moderate	Haemarthrosis	1	3	18	2

1 dose 1 bottle of factor IX

Healing was uncomplicated. This operation was performed under cover of fraction PPSB and plasma transfusions. The patient received altogether 420 ml f fraction PPSB and 4 700 ml of plasma. The factor IX level was kept between 40–20%, during the operation and the postoperative course. This operation has been described in detail elsewhere (1).

Locomotion, hich had been severely impaired before this operation, was now excellent. But Talipes equinus gradually began to recur 1 March 1967 the foot was in moderate (20°) equinus and in July equinus was severe (90°).

1 July 1970 *achillotenotomy + dorsal capsulotomy* was performed under cover of the Swedish factor IX concentrate. A tourniquet as used during the operation. The postoperative course was uneventful. The foot could be placed in about 10° dorsal extension. The plaster was removed after 6 weeks.

Factor IX dosage and plasma factor IX levels (Fig. 1) The patient received 6 bottles of factor IX (3 000 units) immediately before the operation and 3 bottles (1 500 units) 5 h after the operation. The factor IX level ranged between 35 and 70%. No abnormal haemorrhage occurred during the operation. The patient received 4 bottles of factor IX on the first postoperative day 3 on the second, 2 on each of the 3 following days, then 1 bottle a day for 3 days. During this treatment factor IX ranged between 15 and 33%. In the course of the following 6 days he received further 3 bottles of factor IX, which maintained the factor IX level at 6 to 15%. He had no bleedings in the postoperative course.

The patient received altogether 28 bottles of factor IX. No side-reactions were seen.

Case 2

U O male, born 1956, severe haemophilia B (fam. 116). From 7 months of age he had had repeated subcutaneous haemorrhages, bleeding into the muscles of the thigh and into the knee joints. In Feb 1967 Talipes equinus of the left foot was noticed. The defect had gradually developed few years after bleeding into the calf. Physiotherapy failed to produce any effect.

In M y 1969 the patient was subjected to achillotenotomy and dorsal capsulotomy. The foot was in about 45 equinus. After division and elongation of the Achilles tendon the foot could be placed in 10° dorsoflexion. From just below the knee the leg was immobilized in plaster for 5 eeks. No postoperative bleeding. The range of motion of the ankle 6 months after operation was practically normal.

Factor IX dosage and plasma factor IX levels. The patient (weight 40 kg) received 4 bottles of factor IX

Therapeutic result
Regression of haemarthrosis and of pain Cessation of bleeding
Regression of haemorrhage and of pain
Regression of haemarthrosis and prompt cessation of pain Regression of haemarthrosis and prompt cessation of pain Regression of haemarthrosis and prompt cessation of pain Good response. No bleeding
Regression of haemarthrosis and prompt cessation of pain Regression of haemarthrosis and prompt cessation of pain Regression of haemarthrosis and prompt cessation of pain Regression of haematuria for 4 day Some decrease in haemorrhage and pain
Some decrease in haemorrhage and pain
Regression of haemorrhage and pain Regression of haemarthrosis and cessation of pain N bleeding
Regression of haemarthrosis and cessation of pain

(2 000 units) immediately before, and 2 bottles 5 hours after the operation. The factor IX level ranged between 60–78%. No haemorrhage occurred during the operation. On the following 5 days he received altogether 7 bottles. He was given 1 bottle of factor IX on the 7th and 9th postoperative days, during which the factor IX level ranged between 15 and 39%. He had no bleedings in the postoperative course.

The patient received altogether 15 bottles of factor IX.

Case 3

L.P male, born in 1954, with severe haemophilia B (fam. 31). Since 7 months of age he had repeatedly had joint bleeding, mostly bleeding into the knee joints, bleeding into the muscles and haematuria. He was admitted

Table III *Mean values of activity of factor IX VII and P & P in 18 batches of the factor IX concentrat*

	Factor IX	Factor VII	P & P
Units[a]/vial	500 (420–680)	325 (275–375)	275 (200–375)
Units/ml	20 (16–27)	13 (11 15)	11 (8–15)

1 unit activity of 1 ml of normal plasma

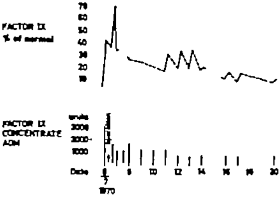


Fig 1 Case 1 B A. fam. 183 23 years old (weight 55 kg). Haemophilia B severe. Achillotenotomy

to his local hospital on altogether 33 occasions between 1954 and 1968. From 1964 he was unable to walk because of contractures of the knees. There was severe atrophy of the muscles of the arms and legs. He could move about at home on tricycle In Nov 1968 he had an episode of bleeding from the right calf and the dorsal part of the right thigh. Spontaneous perforation of the haematoma in the area of the knee-fold and secondary infection, for which he was referred to Malmö General Hospital. Both knees had an extension defect of about 90° (Fig. 2). There was large haematoma extending from the right calf and the dorsal aspect of the right thigh up to the gluteal fold and, below the knee-fold, 10 4 cm ulceration from which oozed old blood and pus (Fig. 3). Culture of the exudate gave growth of Staph. aureus. Roentgen examination showed deformities of the knees.

In Feb. 1969 the lesion of the right leg was incised and revivified. The necroses below the knee-fold were excised and an incision was made high up on the dorsal aspect of the thigh. About 500 ml of clots and pus was evacuated. Devitalized tissue was excised One week later the left leg was immobilized in extension plaster accord-

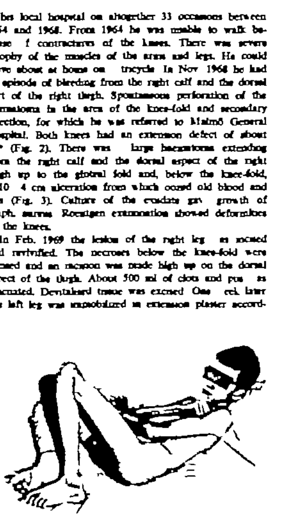


Fig 2 Case 3 Extension defect (>90°) of both knees. Severe muscular atrophy



Fig 3 Case 3. Infected haematoma in right calf and thigh. 10 4 cm ulceration below knee-fold.

ing to the Quengel cast method. Within 4 weeks the extension defect decreased from 90° to 15

I May 1969 the infection in the right leg had healed, leaving only small scab below the knee fold. The right leg was placed in extension plaster according to Quengel. The plaster as changed three times. In Aug. 1969 the extension defect had diminished to 14–15 There was severe subluxation f the tibia backwards. The patient was fitted with bandage and given physiotherapy including exercises in swimming pool, and walking exercises were started. When last seen in July 1970 the patient could walk with knee splint (Fig. 4) When walking out-of-doors he also uses canes.

Dosage f factor IX given and factor IX levels achieved are given in Fig. 5 Factor IX was maintained at levels between 8 and 58%. Bleeding from the wound occurred when the factor IX level fell to 10%. The duration of the effect of the administered factor IX was shorter in this patient than in the other patients studied.

Case 4

L. B., female born 1954, moderate haemophilia B (fam 173). This 16-year-old girl had since early childhood had symptoms of severe haemophilia with repeated joint bleeding, large haematomas, haematuria, and on one occasion a severe retroperitoneal haemorrhage. She had factor IX content of 2%, and normal content of all other coagulation factors as well as a normal Duke and Ivy bleeding time. She is most probably homozygous haemophiliac and has been described in detail lsewhere (11).

The patient was admitted to our hospital in Oct. 1970 because she was pregnant (16th eek). Abortion as strongly indicated on both medical and eugenic grounds.

Since pregnancy was so advanced it was interrupted by hysterotomy (hysterotomia ant. per lap.+exaeresis ovi). Appendectomy was done *en passant*. Operation revealed many extensive adhesions. The margin of the placenta showed signs of frequent previous bleedings. No abnormal bleeding at operation. The postoperative course was uneventful.

Factor IX dosage and plasma factor IX levels (Fig. 6). The patient received 5 bottles of factor IX (2 500 units) immediately before the operation, 2 bottles 3 hours, and further 2 bottles 12 hours, after the operation. The factor IX level ranged between 32 and 69%. She received 3 bottles of factor IX on the first postoperative day and on the second. She then received 1 bottle a day for 6 days and 1 on the 9th postoperative day The factor IX level was kept between 20 and 50% during the first 3 postoperative days and then about 15–20%. She had no bleeding in the postoperative course.

The patient received all together 19 bottles of factor IX. N side-reactions were seen.

Case 5

K. D male, born 1962, moderate haemophilia B (fam. 14). Several primary teeth had severe carious lesions ith periapical destruction. At first sitting 02+ 01+ and +02 were extracted at -day intervals under protection of factor IX concentrate Konyne (Cutter Laboratories). The operations were uncomplicated and the patient was sent home one week after the last traction. N bleeding complications during the later course

Nine months later the patient was admitted for further extractions. Under protection of the Swedish factor IX concentrate −04 and 04+ were extracted. A before, the extraction and the later course were uncomplicated.

On previous occasion −05 had been extracted under protection of plasma, and then the operations had been complicated by bleeding and renal complications producing clinical picture resembling that of nephrosis.

Dosage f factor IX and plasma levels achieved are given in Fig. 7 The factor IX level was kept between 15–50% of normal for 6 days. The effect of the prepara-



Fig 4 Case 3. 10 months later than Fig. . N longer any extension defect. Backward subluxation of tibia on right side valgus of left tibia.

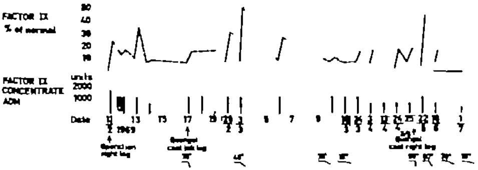


Fig. 5. Case 3. L. P. fam. 31. 15 years old (weight 35 kg). Haemophilia B severe. Operation right leg and extension of both legs. Treatment and course.

tion from Cutter Laboratories was about the same as that from Kabi. No side-effects.

Case 6

A. H., male, born 1938, mild haemophilia B (fam. 177). Several teeth destroyed down to gingival margin by caries and periapical changes. At the first sitting 5+ was extracted. The following day +5, +6 and +7 were extracted surgically and with local plastic bone repair. The wound was sutured and 2 days later −5 and −6 were extracted. After a further 2 days 7+ and 4− were extracted and the cavities were sutured.

The later course was uneventful and the patient was sent home 7 days after the last extraction.

The patient (weight 73 kg) received 4 bottles of factor IX concentrates the first day and then 1 bottle daily for 4 days. The factor IX level ranged between 20 and 33 %.

Case 7

G. G., male, born 1942, severe haemophilia B (fam. 99). Dentition in poor condition with caries of practically all teeth. Several teeth showed roentgenographic signs of periapical bone destruction. 4+ and 5+ were extracted at the first sitting and the alveoli were closed with sutures. No abnormal bleeding. After a further 4 days 4− and 5− were extracted and the cavities sutured. No abnormal bleeding at operation, but profuse bleeding 2 days later. After further suturing and application of local pressure bandage the bleeding ceased and stopped after 2 days.

Factor IX dosage and plasma factor IX levels (Fig. 8). At the beginning of the operation we knew only that the patient had no anticoagulant as judged from the simple anticoagulant test (12). When tested for anticoagulants according to the more detailed method described above, it was later found that the patient had already before the operation a low anticoagulant titre (1 ml of the patient's *plasma inactivated 0.5 unit of factor IX*). At the first extraction the patient was given 1 bottle of factor IX before, and 1 after extraction. The factor IX level rose only to 2 %. On the following 2 days he was given 4 bottles of factor IX and a level of 23 % was achieved. Four days after the first extraction the patient was given 3 bottles of factor IX and 2 further teeth were extracted. The factor IX level rose to 15 %. The patient started to



Fig. 6. Case 4. L. B. fam. 173. 16 years old (weight 51 kg). Moderate haemophilia B. Hysterotomy (abortion) and appendectomy. Treatment and course.

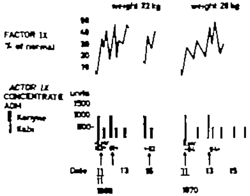


Fig. 7. Case 5. K. D. fam. 14, 8 years old (weight 26 kg). Moderate haemophilia B. Tooth extractions. Treatment and course.

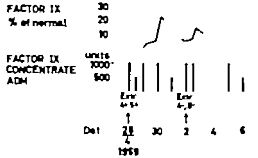


Fig 8 Case 7 G. G fam. 99 77 years old (eight 60 kg.) Haemophilia B severe + inhibitor Tooth extractions. Treatment and course.

bleed after 2 days and then no increase of factor IX was obtained on further administration of factor IX. H was found to have an anticoagulant and, on quantitative determination, 1 ml of his plasma was found to inactivate 4 6 units of factor IX. After 1 month the anticoagulant titre had again fallen to its original level.

DISCUSSION

The observations made in this investigation suggest that the Swedish concentrate is suitable for the treatment of patients with factor IX deficiency One of the advantages of this material is that it is prepared from the supernatant of fraction 1-0 and thereby permits large-scale production without waste of other clinically useful plasma fractions, such as factor VIII The concentrate contains about 20 units of factor IX per millilitre, which means that it has about the same in vitro activity as the French PPSB fraction and the Konyne fraction from Cutter Laboratories. Also in vivo the Swedish concentrate appears to be as potent as the other products. In case 1 for example, achillotenotomy was first performed under cover of the PPSB fraction and then 4 years later under cover of the Swedish concentrate. The clinical response and the yield of factor IX in vivo showed good agreement. In case 5 tooth extractions were first performed under cover of the Konyne fraction and 2 years later under cover of the Swedish concentrate. Also in this case the clinical response and the yield in vivo showed good agreement.

We found the recovery of factor IX in the patients to be 16–69% of that expected (average 38%) This low recovery is consistent with previous observations that the in vivo yield following infusions of plasma or various concentrates is less than that obtained for factor VIII concentrates. When using plasma Biggs (3) found 12 to 36% of expected recovery compared with 14 to 6_% when using concentrate of Bidwell et al. (?). Hoag et al. (8) found the in vivo recovery of the Konyne fraction to be slightly less than half the factor IX activity measured in vitro. Loeliger et al (10) found that only half the dose of administered PPSB was traceable intravascularly Bruning et al. (6) reported the in vivo yield of their factor IX preparation to be about 25% of the in vitro activity Gilchrist et al. (7) gave the in vivo recovery of a commercial preparation from Hyland as about 20–30% The low recovery of factor IX has been explained by the assumption that factor IX is distributed throughout the intravascular and extravascular fluid spaces, whereas factor VIII is retained in the circulation. The poor recovery of activity of both plasma and concentrates in patients with haemophilia B argues against denaturation being of major significance as a cause of the poor recovery of the concentrates.

According to Loeliger et al (10), the biological halflife of factor IX is 20 hours. From our curves it is also apparent that the factor IX level after administration of the concentrate decreased to about half of the level achieved after 24 hours. Loeliger et al. (10) have shown that the turnover rate of factor IX is increased in the presence of generalized hypercatabolism, e.g. fever and anaemia. In case 3 (L. P fam. 31), in which the patient had a large necrosis in the leg and fever and anaemia, the turnover rate was much more rapid than in our other cases.

It is generally believed that the level of factor IX for treatment of a particular lesion is approximately the same as the level of factor VIII necessary in the treatment of a patient with haemophilia A with a corresponding lesion (17). In the two patients (cases 1 and .) who were subjected to achillotenotomy the factor IX level was maintained at levels between 35 and 70% during the operation and on the first postoperative day and then between 15 and 30% for 6 to 8 days No bleeding complications occurred. In case 3 (L. P fam. 31), in which incision with revivification of a giant haematoma with necrosis of the leg was performed, the factor IX level varied between 8 and 30% Bleeding occurred when the factor IX level fell below 10 . In case 4 (L. B. fam. 173), in which laparotomy abortion and appendectomy

were performed, the factor IX level was kept between 32% and 68% during the operation and between 25–50% on the first postoperative day and then for 8 days at a level gradually falling from 30% to 10%. No bleeding at all occurred. Tooth extractions (cases 5, 6 and 7) were not complicated by bleeding when factor IX was kept between 20 and 50% for 5 to 6 days. In case 7 (G. G. fam. 99) with an inhibitor in low titre it was possible to increase the factor IX level at the first extraction to 23% and no bleeding occurred. At the second extraction, which was performed 4 days later the inhibitory titre had increased markedly administration of factor IX produced no increase of factor IX in the blood and the extraction was complicated by profuse bleeding.

Fourteen patients were treated with the concentrate in connection with 71 bleeding episodes (Table II). Most (57 episodes) of these consisted of haemarthrosis. Other manifestations were large haematomas. As a rule these patients were given a single infusion of 1 or 2 bottles of the concentrate (500–1 000 units). The increase in factor IX activity 15 min after injection of concentrate varied from 8% to 40% except in those patients who had inhibitors. The infusion always promptly controlled the symptoms and bleeding did not recur.

Judging from our experience, then, minor haemorrhages can be controlled by raising the factor IX level to 10–20% of normal. This requires a dose of 10–16 units per kg b.wt. At minor operations, such as tooth extractions, good haemostasis can be obtained by maintaining the factor IX level at 20–40% during the operation and then at about 15% for 6–8 days. This requires an initial dose of 20–30 units per kg b.wt. and further doses of 10–16 units per kg b.wt. at 24-hour intervals.

At major operations it is possible to prevent surgical bleeding by maintaining factor IX between 40% and 70% during the operation and on the first postoperative day and then at gradually decreasing levels between 30% and 10% for 8–10 days. This requires an initial dose of 50–60 units per kg b.wt. and of 30–40 units per kg b.wt. per day on the first postoperative days, and then about 10–16 units per kg b.wt. at 24-hour intervals.

No side-effects were seen in connection with the infusions. Four of the patients developed hepatitis, all about 3 months after the infusions of factor IX. Only one of the patients had received blood transfusions besides the factor IX concentrate. The causal relation between the factor IX concentrate and hepatitis thus appears quite certain.

In the patients with inhibitors a certain effect was obtained after administration of large doses of factor IX. After 4–6 days the inhibitory activity increased, and no increase in factor IX was obtained.

Our studies show that the use of Swedish factor IX concentrate is valuable and safe in replacement therapy in haemophilia B.

ACKNOWLEDGEMENT

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STUDIES IN URINARY TRACT INFECTIONS

VI. *Significance of Clinical Symptoms*

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Abstract. Bacteriuria was present in 46% of 742 non-pregnant women referred by general practitioners due to clinical symptoms of urinary tract infection. Impaired urinary concentrating ability and radiological signs of chronic pyelonephritis were most frequently found in patients with previous episodes of fever and flank pain. There was no correlation between the number of previous episodes of lower urinary tract symptoms and current signs of acute or chronic pyelonephritis. Only a minority of patients with laboratory signs of acute pyelonephritis, i.e. temporarily impaired concentrating ability or raised coli antibody titre, showed clinical symptoms of renal involvement. Among placebo-treated patients the clinical symptoms disappeared despite the persistence of bacteriuria, and it is stressed that relief of symptoms does not indicate success of treatment. During the follow-up, 39% of recurrent infections were asymptomatic.

In order to evaluate the significance of clinical symptoms in domiciliary patients with urinary tract infection, past and current symptoms were compared with laboratory signs of chronic and acute pyelonephritis.

MATERIAL AND METHODS

Over a period of 22 months 472 non-pregnant women between the ages of 16 and 65 were referred to the Medical Out-patient Clinic by general practitioners due to urinary tract symptoms. Eighty-four patients had no clinical symptoms at the time of attendance. These patients were referred due to past symptoms of urinary tract infection or because of pyuria revealed by routine urine examination. Patients with serum creatinine above 1.3 mg/100 ml were excluded from the study. Forty of the patients (8.5%) had received an antimicrobial drug within a week before arrival at the clinic, and 28 of these had sterile urine.

The patients were interviewed on the first day by the author about previous and current symptoms of urinary tract diseases. The diagnosis of bacteriuria was established by positive culture from urine obtained by suprapubic bladder puncture, or by the demonstration of more than 10^5 organisms/ml in at least two consecutive mid-stream specimens. Maximum urinary concentrating ability (MCA) was estimated by determination of urinary osmolality 14–18 h after administration of 5 I.U. Pitressin Tannate in Oil (Parke-Davis) (17). Normal concentrating ability > 800 mOsm/kg. Coli antibody titre was estimated by the indirect hemagglutination technique (17, 22). The strain isolated from the patient was used as the antigen. In our laboratory the normal value for reciprocal coli antibody titre is below 1:280.

Intravenous urography was performed in 209 of the 219 bacteriuric women. Clubbing of calyces (but not lesser papillary changes) or difference in kidney size exceeding 10 mm combined with substantial narrowing of parenchyma were recorded as radiological signs of pyelonephritis (6, 7).

In a double-blind trial one-third of the patients received placebo, while the remaining two-thirds were treated for two or four weeks with sulfonamide or antibiotics (13). Urine culture was performed one, two, four and eight weeks after treatment, and thereafter every third month. All patients were instructed to attend the clinic if urinary symptoms occurred during the observation period. Determination of MCA was repeated five or more months after the initial treatment in 115 patients. Improvement in MCA during the follow-up by more than 25% is assumed to indicate that the initial episode was one of acute pyelonephritis, and persistent impairment of MCA in the absence of intercurrent infection is assumed to indicate chronic pyelonephritis (12).

RESULTS

Bacteriuria

Among the 472 women examined bacteriuria was demonstrated in 219 cases. Table I shows the clinical symptoms and urinary findings on admission.

In 21 out of 23 placebo-treated patients in whom bacteriuria persisted for more than one month the symptoms disappeared before the urine became sterile. In 13 of these patients the clinical

Table I. *Urinary findings in relation to previous and present symptoms of urinary tract infection*

	Previous symptoms					Present symptoms					
	None	1 3 episodes of dysuria and frequency	More than 3 episodes of dysuria and frequency	Symptoms of acute pyelonephritis	Unknown	Dysuria and frequency	Dysuria, frequency and backache	Backache only	Fever and flank pain	None	Unknown
No bacteriuria	51	50	73	58	21	91	51	26	15	49	21
Bacteriuria	69	54	45	50	1	103	28	14	38	35	1
% with bacteriuria	58	52	38	46	—	53	36	35	72	42	—

symptoms returned after a period of asymptomatic bacteriuria

Out of 369 recurrences detected, 39% were without clinical symptoms from the urinary tract.

Among 178 patients observed for more than a year recurrent infection occurred within 12 months in 54% of those who had previous symptoms of acute pyelonephritis or more than three episodes of dysuria and frequency compared with 33% of those with fewer previous episodes ($p < 0.01$) (Table II).

Acute pyelonephritis

Clinical symptoms of acute pyelonephritis, i.e. fever and flank pain, were present in 38 bacteriuric patients. MCA was determined in 28 of them and found to be impaired in 26 (Table III) In 18 of these patients the estimation of MCA was repeated after more than five months, and 12 improved their concentrating ability by more than 25% Among the six who did not, four had recurrent infection, and intercurrent attacks of acute pyelonephritis cannot be excluded.

Reciprocal coli antibody titre was determined in 18 patients (including three cases during the follow-up) infected with *E. coli* and with clinical symptoms of acute pyelonephritis. Increased titre was found in 11 cases.

It is generally accepted that fever plus flank pain in bacteriuric patients indicates acute pye-

Table II. *Recurrent infection in relation to clinical symptoms in 178 bacteriuric women observed for more than 12 months*

	Previous symptoms					Present symptoms					
	None	1 3 episodes of dysuria and frequency	More than 3 episodes of dysuria and frequency	Symptoms of acute pyelonephritis	Unknown	Dysuria and frequency	Dysuria, frequency and backache	Backache only	Fever and flank pain	None	Total
Recurrent infection	18	15	20	23	0	30	9	3	15	19	76
Sterile urine during 12 mo.	35	31	19	17	0	56	14	7	13	12	102
% with sterile urine	66	67	49	43	—	65	61	—	46	39	57

lonephritis. In the present series of cases with clinical symptoms of acute pyelonephritis more patients showed impaired MCA (26 out of 28) than raised coli antibody titre (11 out of 18).

Only a minority of patients with laboratory signs of acute pyelonephritis had clinical symptoms of this complaint. Among 35 patients who improved their MCA by more than 25% only 1 had fever and flank pain. These symptoms were present in 11 out of 20 patients with raised coli antibody titres.

Chronic pyelonephritis

MCA was more frequently ($p < 0.05$) impaired in patients with previous symptoms of acute pyelonephritis than among patients without such a history (Table III), while no correlation was found between impairment of the urinary concentrating ability and the number of previous episodes with symptoms of lower urinary tract infection. In 18 patients persistent impairment of MCA indicated chronic pyelonephritis and only six of them had previous clinical symptoms of acute pyelonephritis. However a history of acute pyelonephritis or recurrent symptoms of urinary infections was present in 18 out of 28 patients with radiological signs of chronic pyelonephritis. Radiological signs of chronic pyelonephritis were present in 11 out of 48 with previous symptoms of acute pyelonephritis compared with 17 of the remaining 161 patients without such symptoms ($p < 0.05$).

DISCUSSION

Surveys have shown that most cases of bacteriuria are asymptomatic or clinically unrecognizable (8, 11). Even though many women with urinary tract symptoms do not consult doctor (21) it is nevertheless the clinical symptoms which most often lead to detection and treatment of the infection. The diagnosis cannot, however be based upon the clinical symptoms. Several studies (4 15 16) the present included, have shown that only about one-half of the patients who consult a practitioner due to urinary symptoms do have an infection. Urine culture is therefore necessary in any patient suspected of having urinary tract infection if treatment of persons with sterile urine is to be avoided.

Placebo treatment of bacteriuric patients showed that clinical symptoms tend to disappear despite persistence of the infection, and no definite boundary seems to exist between symptomatic and asymptomatic bacteriuria. This agrees with the findings by Sussman et al. (20), who compared 107 non-pregnant women with asymptomatic bacteriuria with a matched control group. Ninety-one per cent of the bacteriuric subjects gave a past history of urinary symptoms as compared with 63% of the controls.

The findings imply that relief of symptoms does not mean success of treatment. The effect of treatment and recurrences can only be demonstrated with sufficient accuracy by urine culture. The method most suitable for use in general practice seems to be the dip-slide method (1 14).

It is generally accepted that bacteriuria associated with fever and flank pain indicates bacterial invasion of the kidney i.e. acute pyelonephritis. But only a minority of patients with acute pyelonephritis present the clinical feature, and no method suitable for clinical use is satisfactorily sensitive and specific (2, 18, 19).

In the present study MCA was impaired in 26 of 28 patients with clinical symptoms of acute pyelonephritis, and reduction of the concentrating ability thus seems to be a sensitive sign of renal involvement in urinary infections. However Clark et al. (2) found normal concentrating ability in 14 out of 35 patients with upper urinary tract infection demonstrated by culture of urine obtained by bilateral ureteral catheterization.

MCA may be impaired in consequence of previous renal disease, and a subnormal concentrating ability does not establish the diagnosis of acute pyelonephritis. Only improvement by more than 25% in MCA after eradication of the infection can be accepted as a specific sign of acute pyelonephritis (12). Such improvement cannot be expected in all cases of acute pyelonephritis, because MCA might be impaired less than 25% during the acute attack or because full restitution does not occur in all cases. Raised coli antibody titre does not seem to be a sensitive or specific sign of acute pyelonephritis (12, 18). The prevalence of acute pyelonephritis cannot, therefore, be accurately determined in the present material. But the results indicate that only minority of patients with acute pyelonephritis have clinical symptoms of the disease. Similar results were found by Reeves and Brumfitt (18) and Stamey et al. (19).

Table III. *Signs of acute pyelonephritis, chronic pyelonephritis and clinical symptoms*

		Previous symptoms (no. of pats.)				
		None	1–3 episodes of dysuria and frequency	More than 3 episodes of dysuria and frequency	Symptoms of acute pyelo-nephritis	Unknown
MCA	Over 800 mOsm/kg	21	19	12	9	0
	Below 800 mOsm/kg					
	Total	27	25	18	22	0
	Improved > 25 %	11	12	3	9	0
	Not improved, no recurrence	6	6	3	3	0
	Not improved, + recurrence	1	3	8	5	0
	Determination not repeated	9	4	4	5	0
	Unknown	21	10	15	19	1
Reciprocal coli antibody titre	> 1280	4	5	6	4	0
	< 1280	24	24	11	9	0
	Unknown	41	25	28	37	1
Urography	Normal	55	48	34	36	0
	Signs of chronic pyelonephritis	7	3	7	11	0
	Other abnormalities	3	0	4	1	0
	Unknown	4	3	0	2	1

The varied uses of the term "chronic pyelonephritis" make it necessary to state that, in the present work, we use it with reference to the histological changes described by Weiss and Parker (22). From a clinical point of view it is important that the criteria are not specific for the end-result of bacterial infection of the kidney (3). However, the use of more rigid criteria for diagnosing the sequelae of bacterial invasion of the kidney (5, 10) implies a loss of sensitivity (9).

The clinical diagnosis of chronic pyelonephritis in bacteriuric patients should be restricted to those with irreversible functional and/or structural damage of the kidney. Concerning structural changes demonstrated by urography, Hodson (6, 7) has stressed that renal scars without clubbing of the adjacent calyces are not diagnostic of chronic pyelonephritis. This restriction is necessary to distinguish pyelonephritis from ischaemic scars, even though it entails a risk of underdiagnosing pyelonephritis.

In the present study all but three patients with radiological signs of pyelonephritis had impaired MCA, and it is assumed that a normal MCA excludes the presence of chronic pyelonephritis of any clinical importance. Persistent impairment of MCA is considered a sign of chronic pyelonephritis. It may also be caused by recurrent attacks of acute pyelonephritis, but most of such patients probably do have chronic pyelonephritis. It should be added that improvement of MCA after eradication of the infection does not exclude the possibility of chronic pyelonephritis. Several patients improved their concentrating ability by more than 25 % without reaching normal values.

Despite a positive correlation between previous symptoms of acute pyelonephritis and current signs of chronic pyelonephritis, a history with clinical symptoms of acute pyelonephritis does not permit the diagnosis of chronic pyelonephritis. No significant correlation was found between recurrent symptoms of lower urinary tract infection and impaired concentrating ability. Only 16 % of patients with recurrent symptoms had radiological signs of chronic pyelonephritis, and the assumption that recurrent symptoms of urinary tract infection indicate chronic pyelonephritis cannot be maintained.

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Present symptoms (no. of pats.)

Dysuria and frequency	Dysuria, frequency and backache	Backache only	Fever and flank pain	None	Un-know
40	8	5	2	6	0
37	12	4	26	13	0
14	7	1	12	1	0
11	1	2	2	2	0
5	0	1	4	7	0
7	3	1	8	3	0
26	8	5	10	16	1
6	1	1	10	1	0
37	7	8	5	11	0
60	20	5	23	23	1
87	23	9	28	26	0
9	4	2	7	6	0
2	1	1	2	2	0
5	0	2	1	1	1

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Table I. *Plan of treatment*

Susceptibility of the infecting organism	Therapy group	Proportion of pats. in therapy group	Treatment Week 1 2	Week 3-4
Sulfonamide-sensitive	Sulfonamide group	1 3	Sulfametizol	Chloramphenicol
Multiple resistance		1 3	Sulfametizol	Placebo
U known t start of treatment		1 3	Placebo	Placebo
Sulfonamide-resistant	Ampicill group	1 3	Ampicillin	Chloramphenicol
Ampicillin-sensitive		1 3	Ampicillin	Placebo
		1 3	Placebo	Placebo
Sulfonamide- and ampicillin-resistant	Tetracycline group	1 3	Oxytetracycline	Chloramphenicol
		1 3	Oxytetracycline	Placebo
Tetracycline-sensitive		1 3	Placebo	Placebo

less than 15 μg/ml (14) and indicates sensitivity Strains from clean cultures were used and the possibility that some cases are falsely recorded as resistant cannot be excluded. In all cases, however at least two strains were examined separately and the most sensitive were recorded.

Treatment In double blind trial the patients were allocated to "sulfonamide group "ampicill group" or "tetracycline group in accordance with the results f the preliminary sensitivity test as shown in Table I. I *cases of resistance to all three drugs, or if treatment* was started before the results of the sensitivity test were obtained, the patients were placed in the "sulfonamide group Tablets for the first two weeks were distributed in numbered bottles, of which one-third contained placebo tablets only After two weeks the patients were given bottles matched in such way that one-half of the actively treated patients received chloramphenicol for another two-week period and the remaining patients placebo for this period (Table I). The dosage was Sulfametizol g, ampicillin 1 g, oxytetracycline 1 g, or chloramphenicol 1 g, divided into four daily doses.

Definitions. Failure is defined as persistence of bacteriuria with the original organism during treatment, and recurrent infection as the reappearance of significant bacteriuria after period with fewer than 10^4 organisms/ml urine. Recrudescence is recurrence with bacteria of the same species and serotype as found before treatment, while reinfection is recurrence with different species or serotype.

Fewer than 10^4 organisms/ml does not exclude the possibility of bacteriuria. Therefore these definitions imply that some cases in which the urinary tract has not been sterilized are recorded as failures and some as recrudescences. It is difficult to distinguish between failure and recrudescence. Furthermore it is difficult to distinguish between recrudescence and reinfection because cases of reinfection with the same organism will be recorded as recrudescence.

RESULTS

On admission 36% of the 214 patients were infected with sulfonamide-resistant strains. There was no trend to an increased prevalence of resistant bacteria in patients with signs of pyelonephritis, i.e. impaired concentrating ability Resistant strains were found in 34 (37%) out of 92 patients with a maximum concentrating ability below 800 mOsm/kg, and in 22 (36%) of 61 patients with a normal concentrating ability i.e. above 800 mOsm/kg. In 61 patients urinary concentrating ability was not determined.

Selection of resistant strains during treatment

Excluding recurrences after placebo treatment, 327 instances of recurrent infection were detected in 98 patients during follow-up of the 214 women. Of these recurrences 108 were failures or recrudescences and 219 were reinfections. More than two-thirds of all recurrences occurred in 40 patients.

Change from sensitive to resistant strains after sulfonamide therapy occurred more frequently in cases of reinfection than in recrudescences. In patients infected with sulfonamide-sensitive organisms and treated with sulfonamide, resistant strains were found in 24% of failures or recrudescences (Table II) and in 46% of reinfections ($p < 0.01$)

In patients infected with resistant strains no significant difference between sulfonamide-treated and antibiotic-treated patients was found in the frequency of sulfonamide-resistant organisms in recrudescence (84% and 83%) or in reinfection (45% and 51%) (Table II)

After sulfonamide therapy of patients infected with sensitive bacteria 68% of reinfections within the first two months were caused by sulfonamide resistant strains, while only 30% of the reinfections which occurred after more than two months were due to sulfonamide-resistant strains ($p < 0.001$).

Table II. *Sulphonamide-resistant strains in recurrent urinary tract infection*

Sulfonamide susceptibility before treatment	Treatment	Recurrence: Within 2 mo. Total (no.)	Resistant (no.)	Resistant (%)	After more than 2 mo. Total (no.)	Resistant (no.)	Resistant (%)	Resistant of total (%)	Total
Failure or recrudescence									
Sensitive	Sulfonamide	30	7	23	25	6	24	4	
Sensitiv.	Antibiotic	2	1		2	1			
Resistant	Sulfonamide	24	21	88	7	5		84	
Resistant	Antibiotic	11	10	91	7	5		83	
Total		67	39		41	17			108
Reinfection									
Sensitive	Sulfonamide	41	28	68	54	16	30	46	
Sensitive	Antibiotic	9	3		9	2		27	
Resistant	Sulfonamide	28	16	57	23	7	30	45	
Resistant	Antibiotic	31	19	61	24	9	37	51	
Total		109	66		110	34			219

In patients infected with sulfonamide-resistant organisms 57 % of reinfections within two months after sulfonamide therapy and 30 % of reinfec tions after more than two months were due to sulfonamide-resistant bacteria (Table II). The prevalence of resistant strains in reinfections after sulfonamide therapy seems to be rather independent of the susceptibility before treatment.

Susceptibility and outcome of treatment

Of the 214 women 173 were followed for more than 12 months. Patients infected with sulfonamide-resistant organisms tended to have a higher recurrence rate than patients infected with sensitive organisms. In sulfonamide-treated patients 62 % infected with sensitive strains maintained sterile urine for 12 months compared with 55 % of patients infected with sulfonamide-resistant strains (Table III).

Failures plus recrudescence represent the maximum number of cases in which the organisms have not been eradicated from the urinary tract. This was the case in 14 % of sulfonamide-treated patients infected with resistant organisms and in 13 % of sulfonamide-treated patients infected with sensitive organisms (Table III).

The recurrence rate in patients infected with sulfonamide-resistant organisms was nearly the same among patients treated with sulfonamide (45 %) as in patients treated with antibiotics in accordance with the susceptibility test (47 %). In the present study strains with IC50 above 15 μg sulfathiazole/ml are recorded as resistant. Raising this level to 100 μg/ml would not alter the findings, as less than 1 % of all strains had IC50 within the range of 15 to 100 μg/ml.

DISCUSSION

Two important factors that influence the prevalence of drug-resistant strains in urinary tract infections are 1) antimicrobial treatment (1), and 2) the source of the infecting organism (16). These factors probably account for the increased prevalence of resistant strains reported among patients with chronic or complicated urinary tract infec tion (4 10). In the present study of uncomplicated urinary tract infection in domiciliary patients no correlation was found between signs of pyelonephritis (impaired urinary concentrating ability) and the frequency of sulfonamide-resistant organisms.

The influence of antimicrobial agents on the prevalence of drug-resistant bacteria seems to be transient in uncomplicated urinary tract infections. Brumfitt et al. (2) reported that *E. coli* from a selected group of out-patients who had received numerous courses of treatment were remarkably sensitive and that the sensitivity patterns were very similar to those seen in strains from domiciliary patients with acute urinary tract infections.

Bergström et al. (1) found sulfonamide-resistant bacteria in 59 % of recurrences occurring within

Table III. *Course after treatment of urinary tract infection in 173 women observed for more than 12 months*

Sulfonamide susceptibility before treatment	Treatment	No. of pats.	Failure (no.)	Recrudescence (no.)	Reinfection (no.)	Sterile for one year	
						No.	% of all pats.
Sensitive	Sulfonamide	77	1	9	19	48	62
	Antibiotic	*2*	*0*	*0*	*1*	*1*	
	Placebo	35	8	3	7	17	49
Resistant	Sulfonamide	22	0	3	7	12	55
	Antibiotic	19	0	1	8	10	53
	Placebo	18	5	0	5	8	44

two months after 10 days' sulfonamide therapy and in about 40% of recurrences occurring after more than two months. In the present study the change from sensitive to resistant organisms occurred in 68% of reinfections within two months after 14 days' sulfonamide therapy and in 30% of reinfections occurring after more than two months. The two studies are not quite comparable. Ten per cent of the patients in the study of Bergström et al. were infected with sulfonamide-resistant strains prior to treatment, their patients stayed in the hospital for the first two weeks after treatment was started, and no differentiation was made between reinfection and recrudescence.

In the present study sulfonamide-resistant bacteria were found more frequently in reinfection (46%) than in failure or recrudescence after sulfonamide therapy (24%). This suggests that sulfonamides cause a selection of resistant strains in the bowel which, in most cases, is the source of organisms infecting the urinary tract (6, 9, 15). This relationship must be taken into account in the consideration of long-term therapy of urinary tract infections.

It is remarkable that 51% of reinfections following antibiotic treatment of sulfonamide-resistant infections were caused by another sulfonamide resistant organism. Transferable drug resistance (3) probably contributes to the high frequency of sulfonamide resistance in such cases. The findings cannot be explained by selection of resistant strains during treatment.

The issue of clinical relevance is whether the results obtained by treatment of acute uncomplicated urinary tract infections in accordance with the results of sensitivity tests are better than treatment without knowledge of the susceptibility of the infecting organism. Several authors (5, 7, 8) have reported that failure and recurrent infection occur more frequently in sulfonamide-treated patients infected with resistant bacteria than in patients infected with sensitive organisms. A similar tendency was found in the present study. But the findings cannot be explained solely by differences in bacterial susceptibility. The recurrence rate in patients infected with sulfonamide-resistant strains was nearly the same among those treated with sulfonamide (45%) as in those treated with antibiotic in accordance with the susceptibility test (47%). The difference in recurrence rate, previously reported, between patients infected with sensitive and patients infected with resistant bacteria may to some extent be explained by selection of patients with a high recurrence rate. Compared with acute uncomplicated infections, relatively many patients with recurrent infections are infected with resistant strains.

In patients infected with sulfonamide-resistant organisms the frequency of failure and recrudescence was nearly the same among sulfonamide-treated as among those treated with antibiotic in accordance with the susceptibility test. There are several possible explanations for this finding. The material may be biased in two opposite ways. On the one hand, because some strains recorded as resistant with the prediffusion method were sensitive with the tablet method and the patient accordingly was treated with sulfonamide. In such cases the sulfonamide susceptibility is probably higher than in strains recorded as resistant with both methods. On the other hand, patients with bacteria showing multiple resistance were allocated to the sulfonamide group. Such strains are most frequently found among patients with a high recurrence rate.

The main reasons for the good effect of sul-

fonamide treatment of infection with in vitro resistant bacteria are probably the tendency to spontaneous remission in some cases and the fact that the sensitivity tests are poorly adjusted to the pH and the drug concentration obtained in the urine with short-acting sulfonamides.

The study support the view that good response will be obtained by sulfonamide treatment of uncomplicated urinary tract infections acquired outside hospital. A susceptibility test seems necessary only in cases of recurrent infection during treatment or within the first two months in such patients.

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STUDIES IN URINARY TRACT INFECTIONS

VIII. *Escherichia coli O.H serotypes in Recurrent Infections*

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Abstract. A different prevalence of the most common *E. coli* O groups in primary and recurrent urinary tract infections has been reported. In the present study 219 non-pregnant women with bacteriuria were followed up regularly in the Out-patient Clinic after treatment. Two hundred and fifty-five cases of reinfection were detected. Seventy-seven per cent of the infections on admission were due to *E. coli*, as compared to 78% in reinfections. The proportion of infections due to the most prevalent O groups, 02, 04, 06, 075 was considerably higher on admission (56%) than in the reinfections (34%). This was due to some extent to exclusion of cases of recurrence with organisms identical to those found in the preceding infection. However the main reason was a decrease in the prevalence of one serotype, E. coli 02.H4 during the observation period. This was probably caused by changes in the flora from which the urinary tract is infected.

E. coli is the most common urinary pathogen. The presence of O and K antigens and flagellar H antigens makes it possible to subdivide *E. coli* into thousands of stable serotypes. In 80 to 90% of *E. coli* strains isolated from the urinary tract, the O groups can be determined.

A close relationship exists between the serogroups of *E. coli* in urine and faeces from infected individuals (1, 2, 5, 11, 12, 13). The bowel is therefore assumed to be the reservoir from which the urinary tract is infected, even though the possibility of retrograde colonization of faeces with the strain from the urine cannot be excluded in such studies (14).

More than 150 different O groups are known. However a relatively small number viz. 01, 02, 04, 06, 07, 08, 018, and 075 account for the majority of groupable *E. coli* strains in urinary tract infections (2, 3, 5, 6, 9, 11, 12, 13, 14). These O groups are also the most prevalent serogroups in faeces from persons with or without urinary tract infection. Some authors (5, 9, 14) have reported that these O groups are relatively more frequent among *E. coli* strains isolated from the urinary tract than among those from other sources. Such findings suggest a selection of the common O groups because of particular properties which enable them to invade the urinary tract more often than other O groups. However Turck and Petersdorf (12), Jackson et al. (3) and Grüneberg et al. (2) found the incidence of these O groups to be similar to that in faeces from comparable groups of uninfected controls.

In clinical studies, differences have been reported between the distribution of the common O group in the first detected infections and in recurrences. Ørskov and Ørskov (15) and McGeachie (8) found the eight common O groups more frequently in the first detected infections, while Kunin et al. (6) found these O groups more frequently in recurrences. In the present study of women with urinary tract infections the prevalence of the most common O groups found at first attendance is compared with the prevalence in reinfections.

MATERIAL AND METHODS

From Oct. 1966 to July 1968, 219 non-pregnant women aged from 16 to 65 years with bacteriuria were referred to the Medical Out-patient Clinic, Roskilde County Hospital. After treatment the patients were followed-up until May 1970 or until the urine had been sterile for 2 years.

The diagnosis of bacteriuria was established by positive culture from urine obtained by suprapubic bladder puncture, or by the demonstration of more than 10^5 organisms/ml in at least two consecutive midstream specimens (7).

Table I. *Distribution of O serogroups in 339 urinary tract infections due to groupable Escherichia coli*

0 group	No. of infections
2	53
75	37
6	30
4	28
7	18
8, 9, 25, each	12
1, 22, each	9
85	8
11	6
21, 77 each	5
15, 17, 23, 101, each	4
18ab, 83, 88, 123, 127, 16, each	3
12, 18ac, 39, 40, 45, 73, 117, 118, 128, 8/25, each	2
5, X5, 10, 14, 16, 24, 26, 33, 36, 54, 59, 70, 74, 89, 106, 109, 113, 125, 136, 139, 150, 25/35/133[a], 8/60, 25/26, 2/18/25/133/35, 13/18/35, 147/133/129, 7/68, 140/34/13, 18/19/25/26/35/133/147, 78/89, 15/68/125, 14/57, 17/73/125, 5/7, 4/15/125 each	1
Negative in 01–0150	7

[a] A strain reacting in 0 sera 025, 035 and 0133. An examination in existing absorbed sera would not give a definite clearcut 0 group result.

Quantitative urine culture was made on blood agar and lactose agar plates with a calibrated loop, the inoculum being streaked with a sterile glass rod. One colony was selected from each specimen. Where several colonies with different appearance were present in significant number each of these was selected and identified by the Department of Diagnostic Bacteriology, Statens Seruminstitut, Copenhagen.

O and H antigens were determined in all strains of *E. coli* using Escherichia standard test sera, viz. 150 O and 49 H antisera. For details regarding technique, see Kauffmann (4).

In this study reinfection is defined as recurrent infection with an organism different from that found in the preceding infection. Recrudescence is recurrence with identical organisms. Cases of recrudescence are excluded from this study because failure of the treatment cannot be excluded in such cases.

RESULTS

On admission 77% of the 219 women with urinary tract infection were infected with *E. coli*. During the follow-up of these patients 255 cases of reinfection were detected, 78% of which were due to *E. coli*. Sixty-nine different O groups (Table I) and 31 different H groups were found. 106 different O:H serotypes were represented among the 339 infections with O-groupable *E. coli* strains.

Four O groups, 02, 04, 06, and 075 accounted for 56% of all O-groupable *E. coli* strains isolated from the infection on admission (Table II), but only 34% of O-groupable *E. coli* strains in reinfections ($p < 0.001$). There was a slight tendency to a decreased frequency of 04, 06, and 075 in reinfection, while the frequency of 02 was significantly lower among the reinfections ($p < 0.02$).

During the observation period a remarkable decrease occurred in the number of infections due to *E. coli* strains of O group 2 (Table III), whereas the frequency of 04, 06, and 075 was almost un-

Table II. *Distribution of Escherichia coli O serogroups in the infections on admission and in reinfections*

Infecting organisms	On admission N	On admission %	Reinfections No.	Reinfections %
E. coli				
Total	169	77	199	78
0-groupable	151		188	
Rough	17		10	
Not examined	1		1	
Other Gram-negative rods	14	7	33	13
Gram-positive cocci	34	16	22	9
Mixed infections	2	1	1	—
Total	219		255	
E. coli 0 groups				
02	35	23.0	18	9.6
04	15	9.9	13	6.9
06	14	9.3	16	8.5
075	20	13.2	17	9.0
Four common 0 groups	84	55.6	64	34.0
Total 0-groupable	151		188	

Table III. *Distribution of Escherichia coli O serogroups in 336 cases of urinary tract infection during the observation period 1966–1970*

E. coli serogroup	Oct. 1966– Dec. 1967 (%)	Jan. 1968– Dec. 1968 (%)	Jan. 1969– May 1970 (%)
02, total	23.9	10.5	6.1
02. H1	5.7	2.1	3.7
02. H4	13.8	6.3	2.4
02: H7	1.9	0	0
02. non-motile	2.5	2.1	0
04, total	8.0	9.0	6.0
06, total	9.0	6.0	10.0
075, total	9.0	17.0	9.0
Other serogroups	51.0	57.0	68.0
Total no. of infections due to 0-groupable E. coli	159	95	82

Table IV *Distribution of E. coli 02.H4 in infections on admission and in reinfections during the observation period*

	Oct. 1966–Dec. 1967		Jan. 1968–Dec. 1968		Jan. 1969–May 1970	
	First infection	Reinfection	First infection	Reinfection	First infection	Reinfection
E. coli 02. H4 (~)	14	11	6	7	–	2
Total no. of infections due to O-groupable E. coli	115	44	36	54	0	82

changed. Determination of H antigens revealed that the change in the 02 group was caused mainly by 02.H4 which almost disappeared during the observation period.

Table IV shows that the proportion of first detected infections caused by 02.H4 was similar to that found in reinfection in comparable periods.

DISCUSSION

On admission 56% of urinary tract infections due to groupable *E. coli* were caused by 02, 04 06, or 075 which were the most prevalent serogroups. Only 35% of reinfections due to groupable *E. coli* were caused by these O groups. The results suggest different flora in the first infections and in the reinfections. However there are several other possible explanations of these findings.

The exclusion of recurrences with an identical organism results in a lower frequency of the most common serogruops in reinfections. For instance, serogroup 04 was found in 10% of the infections on admission. Assuming an unchanged distribution in reinfections, infection with *E. coli* 04 in both the first and second infections could be expected in 1.0% of all patients. The possibility of two consecutive infections with the same serogroup is probably higher than that calculated if the strain is not eradicated from the bowel during treatment (1 11). Thus exclusion of recrudescence can perhaps explain the lower frequency of *E. coli* 04 06, and 075 in reinfection, but not the decrease from 23 to 9 7% in the 02 group.

If included, recrudescence would create a wrong impression of the prevalence of some serogroups in recurrent infection due to some patients having had many recrudescences because the strain was not eradicated from the urinary tract.

Some degree of type-specific immunity has been demonstrated in experimental studies (10). The possibility of resistance to reinfection with a strain with the same O group due to immunization cannot explain the present findings, because all cases of recurrence with identical serotypes were excluded.

There is a considerable similarity between the *E. coli* flora in the bowel and in the environment (5 11 14), and the distribution of *E. coli* O groups changes constantly (5 13) A possible explanation for the lower frequency of *E. coli* 02 in reinfections might be a change in the distribution of this serogroup during the observation period.

In the present study the proportion of infections with *E. coli* 02 decreased during the observation period, while only minor variations occurred in the proportion of infections caused by 04 06, and 075 The *E. coli* 02 group comprises several serotypes. Determination of H antigens revealed that at least three different serotypes constituted the 02 serogroup in the present study A change in the prevalence of one of these, 02.H4 seems to be responsible for the decreasing frequency of infection due to *E. coli* 02 during the observation period.

It could be demonstrated that the prevalence of 02.H4 was almost the same in infections on admission as in reinfections during the first part of the study During the latter part of the study new patients were not included, but more than one half of all reinfections occurred during this period. It seems most likely therefore, that the different frequency of the common O groups in infections on admission and in reinfections was due to a change in the distribution of *E. coli* 02.H4 in the population.

The epidemological theory agrees well with the findings of a higher frequency of the common serogroups in the first detected infections in some

studies (8, 15) and a higher frequency in reinfections in the study of Kunin et al. (6).

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THE ISCHEMIC LACTIC ACID RESPONSE AND THE MUSCLE LDH ISOENZYME PATTERN IN ALCOHOLICS

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Abstract The blood lactic acid response to anaerobic exercise and the LDH-isoenzyme pattern in skeletal muscle have been studied in 15 acutely intoxicated alcoholics. Three groups were used as controls. Group I healthy subjects, group II patients who had recovered from an acute disease, group III patients examined in connection with an acute disease. A low anaerobic lactic acid response and reduced proportion of LDH 5 together with raised LDH-1, LDH 2 and LDH 3 in skeletal muscle were demonstrated in the alcoholics as well as among the patients ith an acute disease (group III). These changes are interpreted as unspecific findings in skeletal muscle in connection with various diseases.

A low lactic acid production under conditions of ischemic exercise has been reported in alcoholics and considered to give a clue as to the pathogenesis of alcoholic myopathy (1 12) A low rate of glycolysis may however be a consequence of muscle disease as in juvenile muscular dystrophy and neurogenic atrophy (6 13). In acutely intoxicated alcoholics a reversal of the normal glycolytic LDH-isoenzyme pattern in skeletal muscle to a foetal pattern has been observed (10). Such an alteration of the skeletal LDH-isoenzyme distribution is known to be common in diseases of the neuromuscular system (5). Moreover a low lactic acid increase after ischemic forearm work has been reported in thyreotoxic myopathy progressive muscular dystrophy polymyositis and myasthenia gravis (3 8 9) Muscle changes have been observed histologically in infections, in malignant diseases and in various endocrinopathies (2). Approximately 20% of the muscles examined in an unselected autopsy series displayed minor histological disorders (11). There are thus many signs that muscle injury frequently occurs in various diseases.

In view of these findings it is tempting to suspect that the low ischemic lactic acid response in alcoholics is an unspecific finding, reflecting a general disturbance of the muscle metabolism. To test this hypothesis the lactic acid response to anaerobic exercise has been investigated at the same time as the skeletal muscle LDH-isoenzyme pattern in 15 intoxicated alcoholics and in 14 patients with other acute diseases.

METHODS

The muscle biopsy was performed with muscle biopsy needle in the lateral quadriceps femoris muscle. The *LDH-isoenzyme distribution* and the *total LDH-isoenzyme activities* were determined as described earlier (10)

Ischemic muscle test The patient squeezed rolled up inflated blood pressure cuff as often and as hard as possible during 1 min. During this exercise the blood flow to the arm was occluded with help of second blood pressure cuff (systolic pressure 200–300 mmHg) All the tests were conducted by the same investigator in such way that toward the end of the exercise (during the last 5–15 sec) the patients regularly displayed noticeable fatigue and pain, which rendered any further work impossible. Blood samples for the determination of lactic acid were obtained via catheter in an antecubital vein without stasis immediately before the exercise as ell as 1, 3 5 and 8 min after the release of stasis at the end of the exercise. The results are given as the rise in lactic acid above the initial level 1 3 5 and 8 min after the end of the exercise.

Lactic acid was determined according to an enzymatic method (4) The determinations are made on whole blood. Deproteinization with cold perchloric acid was performed immediately after sampling. The Student' *t*-test was used hen the groups were compared.

MATERIAL

The study was conducted on 15 alcoholics (aged 28–63 years) with 5–20 years history of alcoholic abuse, large

Table I. *Distribution of LDH-isoenzymes in skeletal muscle in groups I II and III and acutely intoxicated alcoholics*

The results for the alcoholics include all the muscle biopsies (15 individuals, of whom 13 were investigated 2 5 times). The means for alcoholics on the day of admission differed significantly from group I with respect t LDH-1 LDH-2, LDH-3 and LDH-5 ($p < 0.01$)

	Percentage distribution				
	LDH-1	LDH 2	LDH-3	LDH-4	LDH 5
Group I ($n = 17$)					
Mean	2.6	8.1	19	20	50
Range	0–7.9	0.6–21	3.7–35	4.9–35	22–88
Group II ($= 7$)					
Mean	3.3	8.7	22	19	47
Range	0.7–6.1	2.5–20	15–29	16–25	28–64
Group III ($n = 8$)					
Mean	5 9[a]	17[b]	29[a]	21	27[b]
Range	1.9–14	5.9–34	18–46	15–25	3 1–45
Alcoholics ($= 15$)					
Mean	5.9	17	27	17	34
Range	0–24	1 34	3.5–56	3.5–28	5.7–89

Significantly different from group I ($p < 0.05$).
Significantly different from group I ($p < 0.01$).

quantities of spirits ($^1/_2$–1 l strong liquor) being consumed daily during periods of 1 2 weeks up to 6 months with intervals of abstinence lasting about month. Their admission to the hospital on the present occasion was connected with further bout of drinking. All these patients displayed coarse tremor, perspiration, tachycardia and general anxiety on admission. The following groups were also studied. (In all cases investigated the patients had given their consent to it.)

Group I

Seventeen medical students (aged 22 32 years) and two members (aged 21 and 24 years) of the laboratory staff, all being in good health t the time of the investigation.

Group II

Nineteen hospitalized patients (aged 33–73 years), 13 of whom had been admitted for acute diseases (myocardial infarction, bleeding duodenal ulcer asthma bronchiale, cardiac decompensation etc.) but had recovered or improved by the time of the investigation, while the other six had been admitted for the investigation of anemia, high ESR, nephrolithiasis etc. None of the patients in this group displayed any general effects in the form of fever or weight loss and they were all ambulatory. An ischemic muscle test was made in 16 cases, and muscle biopsy for the determination of LDH-isoenzymes in seven.

Group III

Eighteen patients (aged 18–69 years) all recently admitted for acute diseases. Their general condition was affected, some of them were febrile and they were investigated during the first few days after admission. Examples of the diagnosis in this group were asthma bronchiale, cardiac decompensation, bleeding duodenal ulcer ulcerous colitis, virus pericarditis and acute thyreoiditis. An ischemic muscle test was made in 14 cases, and determination of LDH-isoenzymes in eight.

RESULTS

A low lactic acid response (Table II) and a reduced LDH 5 content in the skeletal muscle with raised LDH 1 LDH 2 and LDH 3 isoenzymes (Table I) were found in the alcoholics and in the patients with other acute diseases (group III) In the alcoholics, who were investigated several times after admission to the hospital, 11 cases showed on some occasion a lactic acid response lower than in healthy subjects, as well as a LDH-isoenzyme pattern which deviated from that in healthy persons. There was, however no significant correlation between the lactic acid response (calculated as the total area circumscribed by the lactic acid curve) and the percental LDH 5 value. Neither was there any significant correlation between the lactic acid response and the total LDH 5 activity per mg wet weight muscle (determined in 11 cases).

Table II. *Rise in lactic acid after anaerobic xercise in groups I II and III and acutely intoxicated alcoholics*

The values for the alcoholics were obtained on the day of admission

Lactic acid response (mM/l)	Minutes 1	3	5	8
Group I (n 17)				
Mean	3.0	2.4	1 7	1.2
Range	1 4–4.9	1.3–3.9	0.7 3.3	0.5–2 2
Group II ($n = 16$)				
Mean	2 5	2.1	1 4	0.9
Range	1 1–4 1	0.9–3.1	0.3–2.3	0–1.6
Group III ($n = 14$)				
Mean	1.8[a]	1 4[a]	0.8[a]	0.5[a]
Range	0.4–3.1	0.3–2.5	0–1.8	0–1.2
Alcoholics ($n = 15$)				
Mean	1.8[a]	1.3[b]	0 8[b]	0.5[b]
Range	0.6–2 8	0.2 3.2	0–3 1	0–1.8

Significantly different from group I ($p < 0.001$).
[b] Significantly different from group I ($p < 0.01$).

DISCUSSION

In this investigation a low lactic acid increase after ischemic forearm exercise was found in acutely intoxicated alcoholics, in accordance with the results of Perkoff et al. (12). However as the lactic acid response was reduced in patients with other acute diseases as well, it is evident that the disturbed lactic acid production reported in alcoholics is an unspecific finding. A low lactic acid response after ischemic work in a subject with an acute disease might be suspected to be due to a failure to cooperate adequately. In this investigation the tested person was instructed to work to exhaustion in order to ensure maximal glycogenolysis. This method of standardization is subjective and a drawback of the method. However all the tests were conducted by the same investigator and on every occasion a marked fatigue and pain were observed. The reversal of the normal "anaerobic" LDH-isoenzyme distribution in the skeletal muscle of the alcoholics and the patients with other acute diseases to a pattern seen in embryonic skeletal muscle may indicate a metabolic dedifferentiation of the muscle and a disproportionate loss in glycolytic capacity (7). Probably the low lactic acid response found in this series reflects a depression of the glycogenolysis in the skeletal muscle, and this change as well as the reversal of the LDH-isoenzyme pattern may represent an unspecific mode of reaction in skeletal muscle in connection with various diseases.

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RESPIRATOR TREATMENT IN COMPLICATED CASES OF ACUTE MYOCARDIAL INFARCTION

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Abstract. Thirteen patients with acute myocardial infarction have been treated during 1968 in a respirator in the intensive care unit at Serafimerlasarettet. Eight of these patients had been transferred from the coronary care unit. The indications for respirator care in the coronary care unit have been frank pulmonary oedema not responding to conventional treatment or marked persistent arterial hypoxia. The usefulness of respirator treatment on these indications is discussed having regard to the fact that none of the patients survived.

In acute myocardial infarction (AMI) treatment in a respirator may be considered for patients who show signs of severe cardiovascular or respiratory deterioration. In the coronary care unit (CCU) of the Department of Medicine of this hospital the indications have been frank pulmonary oedema not responding to conventional treatment, with or without cardiogenic shock, and marked persistent arterial hypoxia. Outside the CCU respirator care has been considered for patients with AMI who have been subjected to extensive resuscitatory measures including external cardiac massage, which is often associated with multiple rib fractures and aspiration of ventricular contents (1).

The purpose of the present study was to report the results that have been obtained using the above indications for respirator care during 1968. It also serves to focus interest on the problem of further reducing the mortality in AMI due to other complications than the arrhythmias, which are those in which the main gains have been achieved.

MATERIAL, METHODS AND RESULTS

During 1968 13 patients with AMI were treated in a respirator in the intensive care unit of the Department of Anaesthesiology. Eight of them were transferred from the CCU where 170 patients were treated for an acute infarction during this period. The other 5 patients came from the Casualty Department or the general wards of the Department of Medicine.

These 13 patients constitute 28% of all respirator patients from the Department of Medicine during the year. The indications for respirator treatment in these 13 patients are shown in Table I.

Endotracheal intubation was carried out with the aid of morphine given i.v. in most patients. No serious cardiac arrhythmias were seen during this procedure. The respirator was of the Engström type. A slow rate was used (1.. min), and in most patients a high insufflation pressure was necessary to obtain a $PaCO_2$ of 30–40 mmHg at a ventilation of 15–20 l/min. In each case pure oxygen was administered during the early phase. As the patient improved, the oxygen concentration delivered was reduced, keeping the PaO_2 between 100 and 150 mmHg. A tracheostomy was not performed until 12–24 h following the endotracheal intubation.

A presentation of these patients grouped according to indication for respirator treatment is given below.

External cardiac massage

This group consisted of 5 patients. One was transferred here after resuscitation at another hospital and was the only one who proved to have irreversible cerebral damage. One of the 5 patients, an 85-year-old man, died after his 32nd defibrillation. Of the other 4 patients 2 survived for about one year. The remaining two patients are still alive two years later.

Therapy-resistant frank pulmonary oedema. Marked arterial hypoxia

The result of respirator treatment on these indications is very discouraging as all 7 patients died. This raises the question whether there is anything to be gained by respirator treatment on these indications. As a background to the discussion all 7 cases will be presented in detail.

CASE REPORTS

Case 1

Male, aged 62, with a history of effort angina and intermittent claudication. Admitted 4 h after the onset of

Table I. *Indications for respirator treatment*

Indication	No. of pats.	No. of deaths
Circulatory arrest + cardiac massage	5	1
Therapy-resistant frank pulmonary oedema	4	4
Low PaO_2	3	3
Stridor	1	0
	13	8

symptoms f AMI. Frank pulmonary oedema on admission and, somewhat later cardiogenic shock. He was given conventional treatment for pulmonary oedema, isoprenaline against the shock and lignocaine because of ventricular tachycardia. Frank pulmonary oedema and shock persisted and respirator treatment was started h after its onset. In the respirator the PaO_2 was low 79 mmHg with 17 l oxygen/min. Several episodes of ventricular fibrillation were defibrillated, finally ending in an intractable asystole on the next day Autopsy showed 75% of the left ventricle to be infarcted.

Case 2

Male, aged 65 with one previous myocardial infarction and effort angina. Admitted because of an AMI. Two days later there was re-infarction. On the next day ventricular fibrillation occurred, which was successfully defibrillated but was followed by frank pulmonary oedema. The pulmonary oedema was resistant to therapy and shock followed. Respirator treatment was started. Ventricular fibrillation alternating with asystole led to death two days later. N autopsy

Case 3

Male, aged 57 with history of hypertension and angina on effort for several years Admitted in frank pulmonary oedema 3 h after the onset of symptoms of AMI. Peripheral signs of shock but systolic BP of 120 mmHg. The pulmonary oedema did not respond to the therapy and respirator treatment was started 7 h after its onset. In the respirator the PaO_2 was 120 mmHg with 15 l oxygen/min. Intensive diuretic therapy was given. While training out of the respirator after one week of treatment the patient sustained re-infarction and developed shock. Several serious arrhythmias developed, and neither transvenous pacemaker nor repeated defibrillation could prevent fatal outcome. The autopsy showed 80% of the left ventricle, and 50% of the right, to be infarcted.

Case 4

Male, aged 65 with history of hypertension and stroke. On admission 1 h after onset of symptoms of AMI the patient was unconscious with signs of shock and frank pulmonary oedema, which did not respond to conventional therapy After the start of respirator treatment a metabolic acidosis was diagnosed with base excess of −11 mEq/l. The blood pressure rose agin, but the patient became oliguric. The respirator treatment could be stopped after one week, and the patient was transferred to general ward, still unconscious and hypotensive. He died 4 days later and the autopsy showed old and recent myocardial infarcts as well as big recent malacia in the left cerebral hemisphere, which probably was the cause of death.

Case 5

Male, aged 49 admitted because of an AMI. He was mentally confused and pulmonary rales were present on admission. The PaO_2 successively decreased to 40 mmHg on the 5th day in spite of 10 l oxygen/min given through pharyngeal catheter Respirator treatment was started, and the PaO_2 values improved. However, the blood pressure successively decreased, the patient developed secondary arrhythmias including ventricular fibrillation and asystole and died after 3 days in the respirator The autopsy showed the infarction to involve 70% of the left ventricle.

Case 6

Male, aged 72, with effort angina for several years. Admitted with normal blood pressure 2 h following the onset of an AMI. Atrial fibrillation developed and was successfully treated by DC shock but later recurred and was treated with digitalis. The PaO_2 successively decreased, and on the 9th day it was 42 mmHg in spite f the administration of 10 l oxygen/min through pharyngeal catheter Respirator treatment was started but the PaO_2 continued to be low The patient went into state of shock and developed frank pulmonary oedema and anuria. He died from ventricular fibrillation after 3 days in the respirator The autopsy showed 65% of the left ventricular myocardium to be infarcted.

Case 7

Male, aged 81. On admission 4 h after the onset of symptoms of AMI he was in shock and had pulmonary rales. On the next day the PaO_2 was 48 mmHg in spite of 11 l oxygen/min administered through pharyngeal catheter Respirator treatment raised the PaO_2 but the patient remained in shock and died on the next day The autopsy showed 60% of the left ventricle to be infarcted.

REMAINING INDICATIONS

Acute stridor is an uncommon complication of AMI, but was the reason for respirator treatment in one patient. Its cause remains obscure (crust formation as a result of aspiration of hydrochloric acid from the stomach or as a result of open mouth breathing?). The patient had been paced because of a complete heart block and was hypotensive before the respirator treatment as well

as during it. The patient quickly improved and is still alive two years later

DISCUSSION

Four of the five patients with long-lasting circulatory arrest and intensive resuscitation manoeuvres could be discharged from the hospital. Whether the results would have been equally good without respirator treatment is of course not possible to state, but it seems probable that these patients did benefit from it, as their resuscitations were complicated.

On the other hand the results were poor when the indications for respirator treatment were frank pulmonary oedema resistant to therapy or very low PaO_2. This poor result should be seen in the light of the fact that 6 of the 7 patients also had cardiogenic shock before or during the respirator treatment, which is a complication carrying in itself a mortality of about 80%.

Smith et al. (2) have reported similar experience: none survived among 5 patients treated with a respirator because of an AMI complicated by gross pulmonary oedema. These two series are small, but raise the question whether it is useful to start respirator treatment on these indications. Two of our patients cleared their frank pulmonary oedema in the respirator but died later on from, respectively another AMI and a stroke. The remaining patients with gross pulmonary oedema improved their PaO_2 but the oedema reappeared as soon as the intermittent positive artificial respiration with high pressure was discontinued. If these temporary gains are to lead to further attempts, it is probably necessary to reduce the delay before starting respirator treatment, which in this series ranged up to 7 h following the institution of conventional treatment.

The extensive infarcts found at autopsy in some of these patients are probably not compatible with life, irrespective of respirator treatment. However the left ventricle commonly showed more than one recent infarct, and theoretically respirator treatment may prevent further infarction if introduced earlier.

The poor improvement of PaO_2 during respirator treatment with pure oxygen again provides evidence of intrapulmonary shunting as an important cause of arterial hypoxia in patients with AMI.

In spite of these negative experiences there may be an indication in the future to treat younger infarct patients with therapy-resistant pulmonary oedema during evaluation for cardiac surgery e.g. infarctectomy and cardiac transplantation.

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ADENOSINEDIPHOSPHATE INDUCED PLATELET ADHESIVENESS IN DIABETES MELLITUS WITH COMPLICATIONS

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Abstract The ADP-induced platelet adhesiveness in 79 patients with diabetes mellitus and 70 healthy controls has been investigated. The mean platelet adhesiveness in the control group was 38% (S.D. 16.7), in patients on insulin 53% (S.D. 15.9), and in patients without insulin 44% (S.D. 13.9). In the group of insulin-treated patients, the mean platelet adhesiveness increased significantly with the number of complications. No correlation between platelet adhesiveness and total cholesterol, blood sugar or dosage of insulin was found.

The importance of the ADP-induced platelet adhesiveness-aggregation for the formation of the haemostatic plug seems well documented. If the platelets are non-reactive to ADP (thrombasthenia) (7) or the reactivity is reduced (von Willebrand's disease) (9, 13) the haemostasis is largely impaired. Since the mechanism of haemostasis and thrombus formation is fairly similar if not identical, it is logical to assume that increased reactivity of the platelets should facilitate thrombus formation and thereby increase the risk of thromboembolic complications. However, the existence of hyperadhesiveness is still discussed, and nobody has proved that such a condition implies an increased risk for thromboembolic diseases, although there is some evidence for this (11).

The purpose of this work has been to investigate the ADP platelet reactivity in diabetes, a condition with increased cardiovascular and thrombotic complications, by measuring the platelet adhesiveness in platelet-rich plasma (PRP) after addition of ADP and to investigate a possible relationship between platelet adhesiveness and the degree of complications. A possible relationship between platelet adhesiveness, blood glucose and total cholesterol was also studied.

MATERIAL AND METHODS

The control group consisted of 70 healthy subjects with mean age of 37 years (S.D. 11.8). The patient group consisted of 79 patients with diabetes mellitus with mean age of 49 years (S.D. 15.6). Fifty-four of these were treated with insulin.

Platelet adhesiveness was measured by the glass bead filter method (7) and performed in citrated rich plasma (8) after addition of a solution of ADP (Sigma Chem. Comp. St. Louis 18, Mo. USA) to give a final concentration of 0.1 μg/ml. The tests were always performed at room temperature immediately after preparation of PRP.

Citrated whole blood. Blood was collected in siliconed glass tubes containing 1/10 volume of 0.11 M solution of trisodium citrate dihydrate.

Citrated PRP. The citrated whole blood was centrifuged at 190 G for 15 min in a refrigerated centrifuge (International-Model PR-2) and the plasma was collected.

Platelet counts were performed with an automatic particle counter (Celloscope 101, AB Ljungberg, Sweden) according to Stormorken et al. (14).

RESULTS

Comparison of normals and the whole diabetic group. The results are given in Fig. 1. The mean adhesiveness in the control group was 38% (S.D. 16.7) and in the patient group 50% (S.D. 15.8) which is significant ($p < 0.001$) (Student's *t*-test). This difference might indicate hyperadhesiveness in diabetics, but could also be due to the age difference in the two groups since the mean age in the control group was 37 and in the patient group 49 years. The latter explanation seems unlikely (see below).

Comparison between insulin and non-insulin treated diabetics. The results are given in Table I showing the number of patients, the mean age and

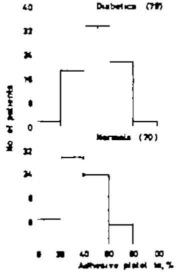


Fig 1 Platelet adhesiveness in 70 normals and 79 patients with diabetes mellitus (54 on insulin and 25 not treated with insulin)

the mean platelet adhesiveness in the two groups. The mean age in the insulin-treated group was 47 as compared to 59 in the other group. In spite of higher mean age in the group without insulin, the mean adhesiveness was 44 as compared to 53 in the insulin-treated group. The difference is statistically significant ($p < 0.01$) and shows that the difference in adhesiveness is not due to age differences.

Relation between platelet adhesiveness and degree of complications The patient group was divided into three subgroups according to the number of diabetic complications. Group I consisted of 39 patients without clinically detectable complications, group II of 16 patients with slight complications from one or two organs (retinopathy nephropathy neuropathy peripheral vascular disease) group III of 24 patients with complications from

Table I. *Mean age and mean platelet adhesiveness in patients on and without insulin (S.D.)*

	No. of pats.	Mean age (y)	Mean platelet adhesiveness (%)	
On insulin	54	47 (14.7)	53 (15.9)	$p < 0.01$
Without insulin	25	59 (12.9)	44 (13.9)	

three or four organs. The results are shown in Table II. It emerges that the mean adhesiveness increased in these groups from 47% in group I to 57% in group III. The differences between normals and these three groups are highly significant ($p < 0.001$). The difference between groups I and II was not statistically significant, whereas the differences between group III and I and III and II were significant. Again it is seen that the difference is not due to age differences since the average age in the three groups decreases with increasing number of complications.

From Fig. 2 it can be seen that the individual values in the different groups overlap.

Comparison between normals and patients without insulin. Whether or not the platelet adhesiveness is higher in patients without insulin than in normals is difficult to evaluate. As seen from Table III, the mean adhesiveness in the normals was 38% and in the patients without insulin 44% and the mean age in the controls was 37 against 59 in the patients. The differences in the mean adhesiveness ($0.025 < p < 0.05$) may therefore be due to the age difference. This suggestion is strengthened by the fact that the 9 normals who belonged to the age group 50–60 had a mean adhesiveness of 44%.

The relationship between platelet adhesiveness and blood glucose Fasting blood glucose and pla-

Table II. *Mean age and mean platelet adhesiveness in normals and insulin-treated patients*

		Mean age (y)	Mean platelet adhesiveness (%)			
Normals	70	37	37.6 (16.7)	$p < 0.01$	$0.01 > p > 0.005$	$p < 0.0005$
Group I	39	53	46.9 (15.4)			
Group II	16	50	48.5 (13.7)			
Group III	24	43	56.7 (16.3)			

telet adhesiveness in PRP were estimated simultaneously in 72 patients. As seen from Fig. 3 no relationship between platelet adhesiveness and blood glucose was found.

Relationship between platelet adhesiveness in PRP and total serum cholesterol. In 73 patients simultaneous recording of plasma total cholesterol and platelet adhesiveness was done. As seen from Fig. 4 no correlation was found between cholesterol and platelet adhesiveness.

Platelet adhesiveness and dosage of insulin. No correlation between dosage of insulin and platelet adhesiveness was found.

Platelet adhesiveness in whole blood. Platelet adhesiveness in whole blood in 70 diabetics had a mean value of 32.4% (S.D. 9.8) and did not differ from the normals. Neither was there any difference between groups I, II and III. This finding is in agreement with previous findings (17), indicating that the liberation of ADP from diabetic red cells is reduced by contact with glass as compared to normals.

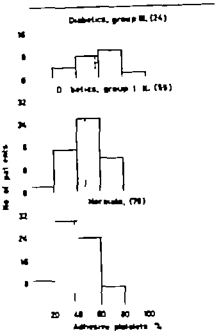


Fig. 2. Platelet adhesiveness in insulin-treated diabetes mellitus with no or few complications (group I + II) and with complication from 3–4 organs (group III).

DISCUSSION

The formation of a white thrombus depends on local factors (extent of vessel injury, blood flow and others) and general factors (plasma coagulation system, the fibrinolytic system, the number and quality of the platelets) which may influence the speed of formation and the magnitude of the thrombus. In diabetes mellitus several investigations indicate an increased activity of the clotting system and the platelet function. Increased Factor I, V and VIII was demonstrated by Egeberg (6) in an unselected group of patients. Valdorf-Hansen (15) showed that Factor VII also was increased, but in patients with complications only. Increased platelet adhesiveness in unselected patients with diabetes mellitus has been demonstrated by numerous authors (4, 5, 12, 17). In selected cases Valdorf Hansen (15) was not able to demonstrate increased platelet adhesiveness in patients without complications or in diabetes mellitus complicated with simple retinopathy only, whereas it was increased in groups with other complications. The bleeding time was, however, shorter than in normals in all groups. His finding is difficult to explain, since a rough inverse correlation between the bleeding time and the number of adhesive platelets has been demonstrated (7, 10). The investigation of Valdorf Hansen indicates that "hypercoagulability" including increased platelet adhesiveness is more or less linked to the complications in diabetes mellitus. This hypothesis is in agreement with the findings of increased platelet consumption in diabetics with complications only (1).

Table III. *Mean age and mean platelet adhesiveness in normals and in diabetics without insulin*

	Mean age (y.)	Mean platelet adhesiveness (%)	
Normals (n = 70)	37 (11.8)	37.6 (16.7)	$0.025 < p < 0.05$
Patients (n = 25)	59 (12.9)	44 (13.9)	

In the present work the platelet reactivity to ADP was slightly higher in patients without complications not treated with insulin than in the control group, but the difference was probably due to the age difference in the two groups. In the insulin-treated group of patients the mean

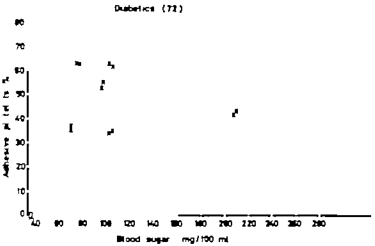


Fig 3 Relation between blood glucose and platelet adhesiveness in 72 patients with diabetes mellitus.

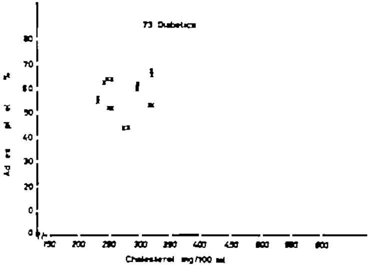


Fig. 4 Relation between total cholesterol and platelet adhesiveness in 73 diabetics.

adhesiveness was significantly higher than in the normals irrespective of complications, but increased with increasing number of complications. This finding indicates but does not prove that the hyperadhesiveness is linked to the metabolic dysfunction in diabetics needing insulin. The finding that the platelet adhesiveness increases with increasing number of complications also indicates but does not prove a causal relationship to the cardiovascular complications, since the increased platelet adhesiveness may be secondary to the complications. The lack of correlation between blood glucose and platelet adhesiveness in the present work is in agreement with the finding of Valdorf Hansen (15) and Bennet (2), but in contrast to the finding of Bridges et al. (5). Valdorf Hansen used the in vivo method of Borchgrevink (3) Bennet the method of Hellem (8) and the in vivo method, whereas Bridges et al. used a modification of the method of Wright (16). The discrepency may therefore be due to technical differences.

ACKNOWLEDGEMENT

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THE ELECTROCARDIOGRAPHIC PATTERN AND MORTALITY RISK IN POST MYOCARDIAL INFARCTION PATIENTS

An 11 Year Follow- p Study

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Abstract. Electrocardiograms of 412 male post myocardial infarction patients have been recorded 1–2 years after their first infarction and classified according to the Minnesota code in groups of possible prognostic significance: A with major Q changes, B with Q and T changes, C mixed group with hypertrophy conduction defects and arrhythmias, and "O" reference group with normal or fairly normal ECGs. The 11 year incidence of subsequent fatal myocardial infarction was significantly increased with pathologic ECG at entry The incidence of sudden death was also increased with myocardial injury ECG pattern. The present study and previous 5-year follow-up study which correlated non fatal as ell as fatal coronary relapses to the same ECG changes seem to justify the following conclusion: The predictive value of the post-myocardial infarction ECG is limited with regard to the risk of suffering new infarct. However, the risk of coronary death seems to be increased when there is permanent ECG evidence of myocardial injury

Electrocardiography has proved to be a useful epidemiologic survey method in large population groups. Recently Blackburn et al. (3), in an international cooperative study on cardiovascular disease, reported 5-year coronary heart disease (CHD) incidence among men free of clinical CHD at entry in relation to minor and nonspecific resting ECG abnormalities. They found that minor T changes, first degree A-V block, and frequent premature beats carried a highly significant likelihood of subsequent clinical CHD They also found that distinct "infarct type Q waves and negative T waves in specified leads carried 10 to 15 times excess risk of CHD death within 5 years. In a previous study (8) we showed that certain ECG changes were of prognostic significance also in survivors of first myocardial infarction. Whereas the 5-year combined incidence of myocardial reinfarction and of new angina pectoris was the same whether or not the entry ECG was abnormal, the incidence of sudden death and of total CHD death was significantly increased when the entry ECG showed major abnormal findings.

The present study correlates the 11 year incidence of death due to CHD with the entry ECG pattern in post-myocardial infarction patients.

MATERIAL AND METHODS

The patients in the study have previously been described in the Oslo diet–heart study (6, 7). That study deals with 412 male survivors of first myocardial infarction, mean age 56 (30–64). A conventional 12-lead resting ECG including the standard leads I, II and III, the unipolar leads VR, VL and VF and V_1–V was recorded 1 2 years (mean 20 months) after the first myocardial infarction. Two of us (J B. and I. H.) coded the ECGs according to the ECG classification system of the revised Minnesota code (10). Each ECG reader read independently of the other and each was unaware of the individual outcome in the follow-up study The coding results of each reader were compared and differences adjudicated in all but 16 records in which third person (P L.) made the decision. The ECGs were then classified in four groups of findings considered likely to have prognostic significance, according to the following criteria.

Group O" ("normal" or reference group) is defined in Table I, and includes miscellaneous items of ST-elevation (code 9-2) in lead V when no other codable items were present, tall P waves (code 9-3), counterclock ise and clockwise rotation (code 9-4-1 9-4-2) and tall T waves (code 9-5).

Group A. Pronounced Q findings (code 1 1 and 1 2) plus any other *additional* ECG findings.

Table I *ECG items in "normal" (reference) group*

Items	Minnesota code
N codable items	0
Axis deviations	2
Minor ST T changes	4–3
	4–4
	4–2 when combined with 5–3
Minor T changes	5–3
Minor ventr conduction defects (low-grade RBBB)	7 3
	7 5
Sinus tachy bradycardia	8–7
	8–8
Miscellaneous items	9–2 (partly see text)
	9–3
	9–4–1
	9–4–2
	9–5

Group B Group A findings plus low-grade Q findings (code 1 3) or coronary" T (code 5-1 5-2) without Q findings.

Group C (mixed group). Ventricular hypertrophy (code 3), pronounced ST or T findings (code 4-1 4-2 and 5-1 5-2), A-V conduction defects (code 6) pronounced ventricular conduction defects (code 7 1 7 2 7-4 7-6) low voltage (code 9-1), arrhythmias (code 8) except sinus tachycardia and sinus bradycardia

It should be understood that some of the abnormal ECGs appear in more than one group. Thus the ECGs with Q changes (code 1 1 and 1-2) will be found both in groups A and B, and T changes (code 5-1 and 5-2) in groups B and C. However the individual cases of the reference group "O" with normal ECGs are distinctly separated from the other groups.

T conclude, groups A, B and, in the main, group C include ECGs with pronounced pathological findings reflecting myocardial injury (groups A and B), and hyper trophy conduction defects and arrhythmias (group C) However, the ECGs without definite signs of myocardial lesions are included in the reference group "O

The present study reports the CHD mortality rates in these ECG groups at the point in time when the last man to enter the study had been followed for exactly 10 years. At this time three-quarters of the survivors had been followed for 11 years. The CHD mortality rates in ECG groups A, B and C have been compared with those in the reference group "O" Tests of significance have been made by χ^2-tests.

Definitions of CHD deaths (described in detail by Leren) (6, 7).

1 Myocardial infarction: (*a*) fatal myocardial reinfarction, (*b*) fatal episodes with chest pains preceding death in which no definite clinical or laboratory diagnosis was established, ( ) fatal heart failure.
2. Sudden death (*a*) witnessed, instantaneous death, (*b*) unwitnessed death.

Table II *ECG groups of possible prognostic significance*

ECG groups	Minnesota code	No.
A (Q)	1 1 1 2	196
B (Q+T)	1 1 1 2 1 3 5–1 5–2	280
C (mix.)	3 4–1 4–2 5–1 5–2 6 7 8 9–1 9–2	61
"0" ("normal")	See T ble I	107

RESULTS

Table II presents the distribution of the ECGs in the four defined ECG groups. Of the 412 ECGs, 67 (16.3%) were without codable items according to the revised Minnesota code. In ad dition, 40 ECGs with minor or insignificant changes previously defined (Table I) were included in the reference group O" making a total of 107 (25.9%) Pronounced Q findings were seen in 196 or 47 6% However by including less pronounced Q waves and the coronary" T elec trocardiographic evidence of myocardial injury was present in 280 or 68% of all ECGs.

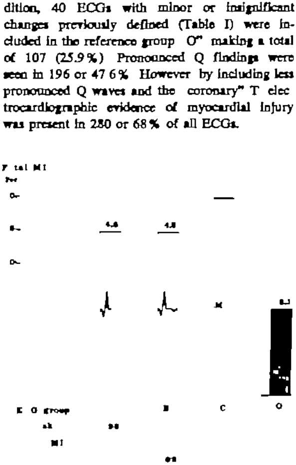


Fig. 1 11-year incidence of fatal myocardial infarction in relation to ECG changes at start of the observation period.

Fig. 1 presents the 11-year incidence of fatal myocardial infarction. A highly significant increase in infarction mortality rate is found in ECG groups A, B and C compared to the reference group with normal ECGs.

The data show also that the incidence of sudden death is significantly increased in ECG groups A and B, which include the ECGs with signs of major myocardial damage (Fig. 2). However, in the mixed ECG group C (hypertrophy, conduction defects and arrhythmias) the incidence of sudden death is not significantly increased.

Finally, in Fig. 3 the total 11 year CHD mortality rate (fatal myocardial infarction plus sudden death) is related to the four ECG groups. Again, a much greater CHD mortality rate is found in all three groups with pathological ECGs at the start of the observation period compared to the reference group.

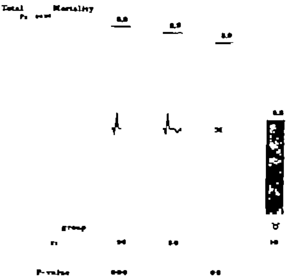


Fig. 3. 11-year incidence of total CHD deaths in relation to ECG changes at start of the observation period.

DISCUSSION AND CONCLUSIONS

Introduction of an ECG classification system, the Minnesota code (2), provided an uniform and clearly defined system for recording electrocardiographic findings. This system has proved useful in population studies of presumably healthy adults (1, 5, 9). In a previous report (8) we were able to show that this system was adequate also in a study of the prognostic significance of certain ECG changes relative to the 5-year incidence of fatal and non-fatal coronary events in survivors of a first myocardial infarction. It was shown that the risk of a new infarction was uninfluenced by the pattern of the ECG findings at entry or whether the ECG was normal. However, the risk of coronary death was significantly increased when the ECG revealed pathologic changes. When evaluating the results of our studies it should be borne in mind that the ECGs were recorded at least one year after the first infarct. Thus the results do not necessarily apply to the immediate prognosis after a myocardial infarction.

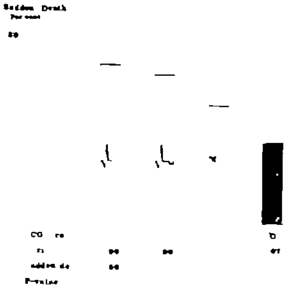


Fig. 2. 11-year incidence of sudden death in relation to ECG changes at start of the observation period.

This study of 11 year incidence of coronary deaths in post-myocardial infarction patients in relation to the entry electrocardiographic pattern confirms the 5-year results. The incidence of fatal myocardial infarction and of sudden death is significantly increased when the ECG at the start of the observation period presents persistent evidence of myocardial injury (Q and T changes in ECG groups A and B) or of ventricular hypertrophy, conduction defects and arrhythmias (group C). There is one exception, the incidence of sudden death is not significantly increased with group C changes. It should be noted that group C includes only 61 ECGs, and therefore we should not put too much weight on this observation.

Table I. *Age and weight pre-treatment and treatment levels of triglycerides (TG) cholesterol (Chol) and free fatty acids (FFA) of the 12 subjects taking part in the study*

Subject no.	Age (y)	Weight (kg)	Before treatment TG (mmol/l)	Before treatment Chol (mg/100 ml)	Before treatment FFA (mmol/l)	During treatment TG (mmol/l)	During treatment Chol (mg/100 ml)	During treatment FFA (mmol/l)
1	21	61	0.63	159	0.74	1.89	180	0.73
2	24	55	0.84	180	1.24	1.21	189	0.61
3	21	54	1.67	200	0.98	2.01	240	0.92
4	25	54	1.19	168	0.86	1.84	150	0.58
5	29	56	0.62	215	0.87	1.32	213	0.91
6	24	50	0.88	165	0.57	1.72	193	1.22
7	27	52	1.14	205	0.98	1.98	212	1.18
8	27	50	0.62	225	—	1.15	262	0.85
9	24	51	0.88	162	1.22	1.86	177	0.71
10	24	65	0.93	183	1.50	1.42	195	0.62
11	22	63	1.06	177	—	2.35	235	0.97
12	22	65	0.68	195	1.31	1.69	258	—
Mean	24.2	56.3	0.92	186	1.03	1.70	209	0.85
S.E.M.	±0.7	±1.6	±0.09	±6	±0.09	±0.11	±10.0	±0.07

After three months of treatment

fat tolerance tests were carried out by injection of fat emulsion (Intralipid® 20% Vitrum, Sweden), the dose was 0.1 g/kg b.wt. Thereafter blood was drawn every 5 min for 30 min. The amount of Intralipid® of the samples was determined by nephelometry (6, 8) using the Thorp nephelometer (20). The results of the fat tolerance tests were given as K_2-values (disappearance rate in %/min) as described previously (7). For each test K_2 was calculated as the linear regression coefficient B in semi-logarithmic plot of Intralipid® levels against time.

One hour after the fat injection, heparin (Vitrum, Sweden), 100 IU/kg b.wt was given intravenously. After 5 and 40 min blood was drawn for determination of the lipolytic activity according to the method of Boberg (4).

Plasma cholesterol and triglycerides were determined according to semi-automatic method using Technicon Autoanalyzer (3 16), plasma free fatty acids (FFA) by titration according to Trout et al. (22). Using preparative ultracentrifuge, the plasma lipoproteins were separated into three major families. The amount of cholesterol, triglycerides and phospholipids in each of the lipoprotein groups was determined as described previously (11). The lipoproteins with density less than 1.006 were called *very low density lipoproteins* (VLDL), those with density between 1.006 and 1.063 were called *low density lipoproteins* (LDL) and those with a density above 1.063 were called *high density lipoproteins* (HDL). Two of the blood samples intended for treatment in the ultracentrifuge were lost. Therefore the results described in Table II and Fig were based on the results obtained from ten of the participants only.

Statistical methods: For all subjects, the difference between each treatment value and the corresponding pre-treatment value was calculated. All differences were expressed as percentage of the corresponding pre-treatment value. Using Student' paired t-test, calculations were performed to determine whether the mean deviations during and after treatment significantly differed from zero.

Table II *Mean value (±S.E.M.) of triglycerides (TG), cholesterol (Chol) and phospholipids (Phos) of the three major lipoprotein families before and after two months of treatment with Anconcene®. The table also gives a statistical evaluation of the individual differences induced by the treatment*

	VLDL TG (mmol/l)	VLDL Chol (mg/100 ml)	VLDL Phos (mg/100 ml)	LDL TG (mmol/l)	LDL Chol (mg/100 ml)	LDL Phos (mg/100 ml)	HDL TG (mmol/l)	HDL Chol (mg/100 ml)	HDL Phos (mg/100 ml)
Before treatment	0.43±0.07	8.2±2.4	12.3±2.0	0.29±0.03	100.3±7.0	67.0±4.5	0.11±0.02	67.2±5.1	119.4±6.6
After 2 months of treatment	0.74±0.10	13.8±1.8	19.1±3.2	0.49±0.04	105.8±9.3	87.2±6.8	0.39±0.03	74.4±7.9	161.6±18.0
p	<0.001	>0.05	<0.05	<0.001	>0.05	<0.001	<0.001	>0.05	<0.05

RESULTS

During the combined treatment all women showed an increase of the plasma level of triglycerides, the mean level being raised from 0.92 to 1 70 mmol/l (Table I). The cholesterol concentration was elevated in ten of the subjects, the mean level increased from 186 to 209 mg/100 ml (Table I). The average concentration of FFA in plasma remained unchanged. During the administration of chlormadinone acetate the mean plasma triglyceride level was elevated during four weeks and the mean cholesterol level during six weeks (Fig. 1).

The mean lipoprotein levels before and during the combined treatment are given in Table II. The concentration of triglycerides significantly increased in all three lipoprotein families. The increase was of a similar magnitude in the VLDL and HDL fractions but was less prominent among the LDL. As the cholesterol and phospholipid concentration of the VLDL increased concomitantly the composition of this lipoprotein group remained fairly unchanged (Fig. 2). This was in contrast to the LDL and HDL fractions, in which the relative concentration of cholesterol was reduced. In the LDL the percentage of both triglycerides and phospholipids increased, in the HDL only that of triglycerides. The increase of triglycerides in the VLDL fraction accounted for about 40% of the total rise of plasma triglycerides. In the HDL the concentration of triglycerides increased more than twice.

The intravenous fat tolerance was unaffected

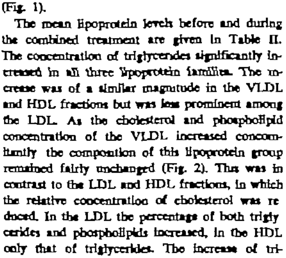


Fig 1 Mean values ±S.E.M. for the concentration of cholesterol (*Chol*) and triglycerides (*TG*) in plasma before, during and after treatment with Anconcene, expressed in per cent of pre-treatment values. and indicate that the values were significantly higher than the pre-treatment values at the 5 1 and 0.1% level respectively Evaluations were done on the individual differences.

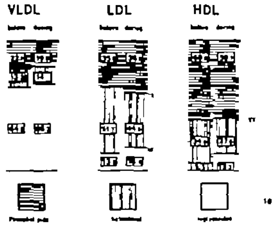


Fig 2. Per cent composition of the three major lipoprotein families befor and during treatment with Anconcene for two months (1 mmol triglyceride = 88.5 mg 100 ml). and indicate that the difference between before and during is significant at the 1 and 0.1% level respectively

by the medication (Table III). During treatment the post-heparin lipolytic activity was unchanged 5 min after the heparin injection but was significantly lowered after 40 min ($p < 0.001$).

DISCUSSION

The results confirmed earlier studies which have shown increased plasma concentration of cholesterol and triglycerides (1 9 14 15 17 23) and decreased post-heparin lipolytic activity (15) during treatment with estrogen-containing oral contraceptives. Larsson-Cohn et al. (17), employing the same agent as used in the present study observed a decrease of plasma FFA. One reason for this difference might be that in the previous investigation blood was drawn at 6 to 7 p.m. while in the present study the samples were taken in the morning after fasting overnight.

It is interesting that the treatment did not change the intravenous fat tolerance. As discussed elsewhere the removal from blood of injected Intralipid®-triglycerides is very similar to that of chylomicrons (5 10) Available evidence suggests that the mechanisms responsible for the removal from the blood of chylomicrons and VLDL are the same (19). It is thus possible that the intravenous fat tolerance test reflects the removal of VLDL-triglycerides (5) On that assumption the

Table III. *The intravenous fat tolerance (±S E.M) and the post-heparin lipolytic activity (PHLLA) before and after two months of treatment with Anconcene®*

	Before treatment			During treatment		
		PHLLA			PHLLA	
Subject no	K_2 (%/min)	(µmol glycerol/ml/min) 5	40′	K_2 (%/min)	(µmol glycerol/ml/min) 5	40′
1	4.8±0.3	0.023	0 038	7 0±0.6	0.010	0 034
2	5.8±0.4	0 012	0 076	5.5±0.7	0 015	0.055
3	7.0+0.8	0.006	0 056	8.9±0.9	0 005	0.030
4	7.2±0.6	0 015	0.058	—	0.004	0.005
5	9.6±0.5	0 026	0.071	8.9±0.2	0.007	0.051
6	6.7±0.9	0.020	0.075	8 4±0 3	0.022	0 033
7	6.8±0.4	0.023	0.057	5.6±0.1	0.011	0 014
8	15.9±2.5	0.093	0 111	16.5±3.8	0.035	0.106
9	6.9±0.8	0.010	0.034	12.9±1 0	0.011	0.031
10	8 4±0.1	0.034	0 089	6.5±0.4	0 066	0.070
11[a]	*6.7±0.5*	*0 023*	*0 053*	*6 9±0.3*	*0.017*	*0.038*
12[a]	*11.4±0.4*	*0.027*	*0.064*	*9 1±1.2*	*0 015*	*0.038*
Mean	8.1	0.026	0 066	8.7	0.018	0 042
S E.M.	±0.9	±0 007	±0.006	±1.0	±0.005	±0.008

After three months of treatment.

present results suggest that the clearance of VLDL-triglycerides from plasma was not affected by the treatment. This would mean that the hypertriglyceridemia was due to an increased inflow of triglycerides into the plasma, i.e. an increased turnover rate. As the liver appears to be the major site for the production of plasma triglycerides this would imply that Anconcene® stimulated the hepatic secretion of plasma triglycerides. It has been suggested that by stimulating the hepatic triglyceride synthesis, raised insulin levels may cause hypertriglyceridemia (18). Therefore it is interesting that oral contraceptives have been found to raise the basal plasma levels of immunoreactive insulin (15 21).

The fact that during treatment with chlormadinone acetate the hypertriglyceridemia decreased, but that raised values were found for several weeks, indicated that Anconcene® caused a change of some metabolic pathway and that this alteration persisted for some time after the treatment. Although a certain degree of additive or synergistic action between the estrogen and the gestagen cannot be completely excluded, it seems that the estrogenic component was mainly responsible for the alterations. This is well in agreement with the observation of Beck (2) that elevated plasma levels of triglycerides and cholesterol during pregnancy and during treatment with estrogen-containing oral contraceptives remained raised for about six weeks, while 0.5 mg chlormadinone acetate daily induced no changes.

It is noteworthy that although the results of the intravenous fat tolerance tests suggested an unchanged removal rate of triglycerides from plasma, the lipolytic activity 40 min post-heparin was reduced. The lipolytic activity as measured in the present study is probably mainly due to the lipoprotein lipase released by the heparin. Lipoprotein lipase is believed to be necessary for the removal of VLDL-triglycerides from plasma (19) There are several possible explanations why the fat tolerance did not decrease in parallel with the post heparin lipolytic activity First of all it has not yet been shown that post-heparin plasma lipolytic activity reflects the functional plasma triglyceride removal rate except in Type I hyperlipoproteinemia of Fredrickson et al. (12) in which this activity is very low and plasma triglyceride levels very high. It is also possible that the activity 5 min after the heparin injection, which in this study was not significantly lowered, is a better indicator of the triglyceride removal capacity than that after 40 min. In this context it may be mentioned that in previous studies we have not been able to demonstrate any correlation between the intra-

venous fat tolerance and the lipolytic activity (unpublished data).

The fact that the lipid composition of the VLDL family was relatively unchanged suggested an increased synthesis of normal VLDL molecules.

The results of the present study only partially agreed with the findings of Furman et al. (13). These authors reported that estrogens induced a relative decrease of the cholesterol content of the LDL and HDL fractions, while the percentage of triglycerides was fairly unchanged. One explanation to this discrepancy could be that in the present investigation all subjects served as their own controls, while in the study of Furman et al. the results obtained in three women on estrogen therapy were compared with those from 13 control subjects.

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THE EFFECT OF DIFFERENT DIGOXIN DOSES ON SUBJECTIVE SYMPTOMS AND PHYSICAL WORKING CAPACITY IN PATIENTS WITH ATRIAL FIBRILLATION

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Abstract In a dose-response study 14 patients with atrial fibrillation have been treated with increasing digoxin doses, 0.125, 0.25, 0.375, 0.50, 0.75 and 1.00 mg daily until toxic symptoms or signs appeared. Dosage was changed every 14th day. The size of the doses was unknown to the patients. In this paper the effect on subjective well-being and physical working capacity is presented. The mean "optimal" dose was 0.44 mg/day varying from 0.25 to 0.75 mg/day. An improvement was, however, achieved also at lower digoxin doses. The increase in physical working capacity measured on a bicycle ergometer was less impressive than the improvement in subjective well-being and in physical capacity observed by the patients in their daily activities. Mean toxic dose was 0.65 mg/day. The toxic symptoms and some reasons for the great individual differences found in digoxin tolerance are discussed.

Digitalis has for nearly 200 years been the basic drug in the treatment of heart disease, but still there are no methods for adequate control of the treatment (14, 36). Consequently digitalis intoxication remains a great problem. The difficulties in digitalis treatment are reflected by the remarkably great variations in average maintenance dose of digoxin recommended—from 0.25 mg (13) to 0.75 mg/day (19).

Digitalis effects in man have been evaluated from changes in the ventricular rate in atrial fibrillation, signs of increased myocardial contractility, improvement of congestive heart failure and changes in the ECG (11, 19, 20, 21, 42, 50, 54, 57, 58). However, all these methods have limitations, especially the last (18, 36, 43, 50). A better knowledge of the clinical effects of different doses of glycosides is of importance, especially nowadays when the question of the intensity of digitalis treatment must be seriously discussed in view of the reported increase in the frequency of digitalis intoxication (12, 15, 30). Remarkably few attempts have been made to study the dose-response relationship of digitalis treatment (4, 17, 19, 39, 50).

The purpose of the present investigation was to evaluate the effects of a wide range of digoxin doses on subjective symptoms and physical working capacity in patients with atrial fibrillation. The effects on the ventricular rate at rest, during exercise tests and during the patients' daily life will be reported separately (44, 45), as will the relation between the clinical effects of digoxin and the plasma digoxin concentration measured by a new method (6, 46).

SUBJECTS

The investigation was carried out on 20 patients with atrial fibrillation. In the main dose-response study nine men and five women were investigated. The other patients, four men and two women, were, without being informed about it, used as controls.

Patients in the main dose-response study

The mean age of the patients (Table I) was 54.9 years, range 32–68, those with rheumatic valvular heart disease (RHD) being somewhat younger than the others, 51.7 and 58.1 years, respectively. The mean weight during the investigation (Table I) was 75.9 ± 11.1 kg (mean ± S.D.). Patients with RHD were lighter, 66.4 ± 3.9 kg, than the patients without RHD, 85.3 ± 6.8 kg.

Diagnoses (cf. 1) and case histories are given in Table I. Seven patients (nos. 1–7) had *rheumatic valvular heart disease* diagnosed from findings at physical examination, phonocardiogram, ultrasoundcardiogram, chest radio-

Table I. *Diagnoses, short histories and some other clinical information on patients in the main dose–response study*

Weight is given as mean values from all examinations
AF = atrial fibrillation, AI = aortic insufficiency AS = aortic stenosis, ASHD = arteriosclerotic heart disease, digit. = digitalized, CS = cardiac symptoms, HCVD = hypertensive cardiovascular disease, HT = hyperthyroidism, IAF = "idiopathic atrial fibrillation, MI = mitral insufficiency MS = mitral stenosis, MSop = mitral commissurotomy RF = rheumatic fever RHD = rheumatic valvular heart disease.

Pat. no.	Sex	Age (y)	Weight (kg)	Height (cm)	Diagnoses (y of diagn.)	Short history	Duration of AF (y)
1	♀	57	63	161	MI + MS (1967)	RF in adolescence and 1934 CS 1967 not digit.	1/12
2	♂	49	71	182	MSop + MI (1961)	Susp. RF as child. Cerebral embolism 1961 digit., operated and improved, later slowly worse	3/12[a]
3	♂	32	63	171	MI + MS (1964)	CS 1967–68, digit. 1968, improved	1/12[a]
4	♂	59	72	169	MI (1967)	RF 1958 digit. 1967 improved	2 or more
5	♂	51	70	165	AS + AI + MS (1967)	CS since 1958, digit. 1967 slowly worse	1½
6	♀	52	65	155	MS (1967)	RF 1932 and 1951 CS last years. Digit. 1967	2 or more
7	♀	62	63	159	MSop (1960)	RF 1934. In 1960 arter. embolism right leg, digit., MS op., improved	6
8	♂	62	88	165	ASHD (1962)	AF with card. decomp 1959 digit., improved. Since then some episodes of chest pain	8
9	♀	61	76	167	HCVD (1968)	Infectious disease, AF and card. decomp. 1962, digit., improved	5½
10	♀	50	89	170	IAF (1967)	AF discovered by chance 1967 digit., slightly improved	2/12 or more
11	♂	56	87	182	IAF (1955)	AF since 1955 digit.	13[a]
12	♂	49	96	181	IAF (1968)	AF discovered by chance 1968, digit., no obvious effect	?
13	♂	68	78	182	HT + ASHD (1969)	AF discovered by chance. Since 1966 slowly deteriorated. Not digit.	3 or more
14	♂	61	83	173	HCVD (1969)	AF discovered by chance. Not digit.	3/12[a] or more

[a] Previous brief episodes of AF

gram (Table II) and in most cases heart catheterization. T patients presumably had *arteriosclerotic heart disease* (ASHD). Before digitalization patient 13 had effort angina, no. 8 had previously had episodes of severe chest pain. I both patients ECG showed left axis deviation (< −30°), which is usually due to ASHD (3, 23). Patient 13 also had moderate *hyperthyroidism*. Protein-bound iodine was 12.3 μg/100 ml, T3 resin test 158% and basal metabolic rate +46%. No treatment against the hyperthyroidism was given before the present investigation was finished. *Hypertensive cardiovascular disease* (HCVD) was diagnosed in patients 9 and 14 showing slight hypertensive changes, grade *I–II*, in the eye grounds (31). BP taken under basal conditions are shown in Table II. At the Out-patient Department values up to 205/105 and 200/120 mmHg, respectively were registered. The remaining 3 patients had no signs of cardiac or extracardiac diseases of etiological importance for atrial fibrillation, and were classified as having *idiopathic atrial fibrillation* (IAF).

Patients in the control study

Six patients constituted control group (Table III). They had the same mean age, 54.8 years, range 39–62, and the same mean weight, 75.9 ± 7.0 kg, as the patients in the main group. Three controls had RHD the others IAF. Their physical activity and capacity were well comparable to those of the patients in the main group.

PLAN OF THE INVESTIGATION

Digoxin treatment in the main dose–response study

Patients in the main group were treated with series of different digoxin doses for 2–4 months (Fig. 1). Three patients had not been given digitalis before (Table V), whereas the others had been on continuous digitalis treatment for an average of 4 years, range 1 month–10 years, and had received unchanged digoxin maintenance doses for at least 14 days (mean 10 months).

For ethical and practical reasons the digoxin therapy

Table II. *Physical activity, symptoms, clinical grade according to the classification of N Y H A and heart volume at the beginning of the main dose–response study*

In patients not earlier digitalized clinical grade after digitalization is shown in parentheses. Blood pressure is given as mean values from all examinations

D = dyspnea, N = nausea, P = palpitation, T = tiredness.

Pat. no.	Physical activity	Symptoms	Clinical grade	B.P (mmHg)	Heart vol. (ml–ml/m² BSA)
1	Housework, berry-picker in summer. Sick-listed, daily walks	D T	III A (→ II)	135/85	1 140 – 690
2	Chauffeur with loading work. ½ sick-listed	D T Somewhat anxious	III A	115/80	1 280–670
3	Foundryman. Sick-listed, moderate exercise	D T	II	105/70	1 210 – 690
4	Building job	T	II	135/85	1 190 – 660
5	Pipelayer at shipyard. Sick-listed, daily walks	D T	III A	125/80	1 210 – 700
6	Housework. Daily walks	D T Hacking cough	III A	125/85	1 120 – 700
7	Housework, some office work" at home. Daily walks	D T N	III A	135/80	1 030 – 640
8	Warehouse worker. Some gardening. ½ sick-listed	D T P	III A	130/80	1 480 – 770
9	Office work, daily walks	Sometimes headache	I	175/100	1 050 – 570
10	Housework at farm. Serving at schoolmeals	D T	II	140/90	850 – 430
11	Agricultural work	T D Somewhat depressed	II	120/80	1 030 – 490
12	Agricultural work	—	I	120/85	1 010 – 460
13	Pensioner. Some joinery work, daily walks and gardening	D T P Effort angina	III A (→ II)	140/80	1 330 – 670
14	Agricultural work	D T	II (→ I)	155/110	1 090 – 550

was not quite withdrawn for the already digitalized patients; it was only reduced to 0.125 mg daily. For the same reasons placebo was not used. From period II all patients were treated with the same, systematically increased digoxin doses, 0.125 0.25 0.375 0.50, 0.75 and 1.00 mg/day (Fig. 1), until manifestations of digitalis intoxication appeared (patients 8 and 9 also received 0.625 mg/day). Dosage was changed every 14th day. Digitalis intoxication was diagnosed when gastro-intestinal, cardiac or other symptoms or signs indicative of toxicity developed and then rapidly disappeared when the digoxin dose was reduced. In some uncertain cases the dose was afterwards repeatedly changed between nontoxic and suspect toxic dose level to ascertain whether side effects were due to the digoxin treatment or not.

The patients were informed that the purpose was to study the individual reaction to different digitalis doses. New and older methods for estimating the clinical response to the drug would be tested in order to find the most favourable dose for each participant. Patients already digitalized were informed that this optimal" dose could be lower or higher than the original maintenance dose and that first lower and then higher doses would be tried. The size of the dose changes was unknown to the patients and was randomized to reduce possible bias. Digoxin tablets in different colours were specially prepared containing 0.125, 0.25 0.375 and 0.50 mg of digoxin (AB Draco, Lund, Sweden). Up to daily dose of 0.50 mg the patients received one tablet day and at higher doses combinations of two tablets. In that way the patients did not know which dose they were given in the different treatment periods, how small the re-

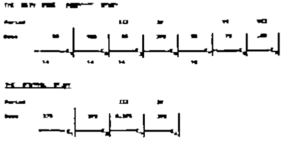


Fig. 1. The plan of the investigation. *E* examinations at the end of the 14-day treatment periods.

Table III. *Some clinical data for the 6 patients in the control study Clinical grades are assessed according to the classification of N Y H A, as modified by Bishop and Wade (8)*

AF = atrial fibrillation, AI = aortic insufficiency IAF = "idiopathic" atrial fibrillation, MI = mitral insufficiency MS mitral stenosis, MSop = mitral commissurotomy (in parentheses year of operation), D = dyspnea, P = palpitation, T = tiredness.

Pat. no	Sex	Age (y)	Weight (kg)	Height (cm)	Diagnoses	Duration of AF (y)	Physical activity	Symptoms	Clinical grade
15	♂	60	80	179	IAF	1½	Porter with heavy work	T D some times P	II
16	♂	52	84	183	MSop (1957) + AI	1	Welder and machine operator	T D	III A
17	♂	39	67	182	MSop (1963) + MI	4/12*	Publicity work and chauffeur with loading work	—	I
18	♂	62	82	182	IAF	3/12*	Agricultural work. ½ sick-listed	T D	II
19	♀	62	73	172	MSop (1963)	10 or more	Housework, daily walks	T D	III A
20	♀	54	70	166	IAF	5/12*	Housework, daily walks	D	I

* Previous brief episodes of AF

duced dose was, nor when they received their previous maintenance dose again. At the end of each treatment period the number of tablets consumed by the patients was checked.

During the investigation 8 of the patients were treated with bishydroxycoumarol. No diuretics were given.

Procedure in the control study

The possible non-specific effects of the repeated examinations involving increased familiarity with the equipment and the investigator possible training effect, and the anticipated effects of supposed changes of the digoxin dosage were studied on the 6 control patients. They were told about the purpose and the design of the investigation in the same way as the patients in the main group. At the beginning of the investigation the controls had been on digitalis treatment for an average of 6 years and on an unchanged maintenance dose of 0.375 mg digoxin daily for at least 14 days (Fig. 1, period I), on the average for 11 months. Also for these patients tablets in different colours were specially prepared (AB Draco), all however containing 0.25 mg digoxin. Of these different preparations 1 / tablets daily were administered in three 14-day periods (period II–IV). Thus, during the whole investigation the controls continued on their previous digoxin maintenance dose, 0.375 mg daily without knowing it.

Examination routine

At the beginning of the study the history of each patient was taken and careful physical examination performed. ECG at rest and at exercise, phonocardiography ultrasoundcardiography (16, 24) and X-ray examination of the heart were done in conventional ways, and blood samples for number of laboratory tests were drawn. A continuous ECG during 8–10 h of the patient daily life was also recorded by means of portable tape recorder (45).

At the end of each treatment period new examination was performed (Fig. 1), including an exercise test. All examinations were made by the author starting at the same time in the morning, about 2 h after the patients had eaten standardized light meal. On the day before, an ECG for 8–10 h was recorded just as t the first examination.

The controls were examined in exactly the same way as the other patients.

Comments on the plan of the investigation

When clinical effects of different maintenance doses of digitalis glycosides are to be tested, each treatment period should be long enough for reliable steady state to be reached. The desirability of long treatment periods has to be weighed against the practical disadvantages of very prolonged investigations. In earlier clinical investigations periods of 1 to 8 weeks have been used (4, 19 21, 50). Marcus et al. (38), however, found that the concentration of radioactivity in blood reached plateau already on the sixth day of treatment, when 0.5 mg of tritiated digoxin daily was administered to previously undigitalized normal subjects. Jelliffe (28) showed body digoxin stores to be in kinetic equilibrium about eight days after change of the digoxin dose in patients with normal renal function. According to these results the 14-day treatment periods chosen in the present study should be adequate. Furthermore, the small successive increments in dosage would favour steady state conditions. The adequacy of 14-day periods was further supported by the fact that changes of the subjective well-being mostly were fully apparent within 5–7 days after shift of the digoxin dosage.

BP (mmHg)	Heart volume (ml – ml/m² BSA)
130/85	1 170 – 590
120/80	1 390 – 670
115/85	1 160 – 600
120/85	1 160 – 530
155/95	990 – 540
135/85	780 – 450

It may thus be concluded that the major part of clinical and hemodynamic effects of digitalis treatment is revealed during treatment periods of 14 days. This does not exclude that under certain conditions a non-steady state may be present for a longer time (4, 19). Thus, even at supposed kinetic equilibrium of the glycoside some patients may show a continuously increasing cardiac decompensation when insufficient digoxin doses are given. This actually happened with patient 3 in this study when the digoxin dose was reduced to 0.125 mg daily. On the other hand, at doses near the toxic limit a slow cumulation of digoxin may result in digitalis intoxication after a fairly long time of unchanged digitalis treatment. This probably occurred in some control patients who during the last treatment period developed slight gastro-intestinal disturbances.

The digoxin doses were systematically changed and the size of the doses was unknown to the patients. A double blind study with the doses mixed at random (19) was not found to be suitable. If periods with very different doses had been mixed, the results could be variably influenced by the previous dose. It was also impossible to know in advance the number of doses to be tested. Furthermore, systematic changes of the doses facilitated a comparison of the effect of different digoxin doses on the subjective well-being. A treatment plan similar to the usual clinical practice was also considered preferable (50).

Intercurrent diseases may be a problem in a prolonged investigation of this type. Three patients suffered from short episodes of slight upper respiratory infections and one patient had diarrhoea for one day. This caused a lengthening of a few treatment periods, but did not seem to influence the results. All patients were instructed to change neither their physical activity nor their smoking habits during the investigation.

METHODS

A thorough *history* was taken at the first examination, and was used as a basis for grading the disability of the patients according to the criteria of the New York Heart Association (N.Y.H.A.) (41) as modified by Bishop and Wade (8). At the following examinations the patients gave an unprompted account of their activity and symptoms during the period. According to a specially prepared questionnaire they were then asked about the different symptoms at the various degrees of exertion. They were also asked to compare the current period to the previous periods. *This subjective account could often* be supplemented by information from relatives of the patients.

Exercise tolerance tests were performed on an electrically braked bicycle ergometer (Elema-Schönander AB, Sweden) (26) with the patients in sitting position according to the methods described by Sjöstrand (51, 52) and Wahlund (56). The same bicycle, calibrated once a year, was used during the whole investigation. The patients worked continuously for 6 min on each work load, women at 100, 200, 300 etc. kpm/min, men at 150, 300, 450 etc. kpm/min. The tests were interrupted when the patients could not continue because of tiredness and dyspnea, never because of increasing number of extrasystoles. Chest pain was not provoked and high ventricular rate was not considered a reason to discontinue the tests. The *physical working capacity* (PWC) was estimated as the highest work intensity which the patients could maintain for 6 min. If a patient was able to work for some minutes also on the next work load, PWC was proportionately elevated (22). A patient who managed 450 kpm/min in 6 min and 600 kpm/min in 2 min was thus considered to have a PWC of

$$450 + \frac{2 \times 150}{6} \text{ kpm/min, i.e. } 500 \text{ kpm/min}$$

Systolic and diastolic *blood pressures* were recorded by indirect measurement before the exercise tests when the patients had rested in supine position for about 1 hour. Indirect systolic blood pressure was also measured towards the end of each work load.

Chest X-ray examination was performed in postero-anterior and lateral projections with the patients standing. Barium contrast in the esophagus was used to outline the posterior border of the heart. Heart volume was calculated according to the method of *Jonsell* (29).

At *spirometry* the vital capacity (VC), forced expiratory volume in one second ($FEV_{1.0}$), $FEV_{1.0}$ in per cent of VC (FEV%) and the maximum voluntary ventilation at a free breathing frequency (MVV) were determined with a Bernstein spirometer (Kifa AB, Sweden).

The following *laboratory tests* were made: ESR and Hb (estimated at every examination), sodium, potassium (estimated at every examination), chloride, calcium and magnesium in serum; protein-bound *iodine* and *T 3* resin test, bilirubin, thymol reaction, alkaline phosphatase, GOT, GPT, LDH, serum protein electrophoresis and retention of bromsulphalein (BSP) 30 min after an intravenous BSP injection; creatinine in serum and 24 hour creatinine clearances on three different days.

Table IV *Physical working capacity (kpm/min) at different digoxin doses and changes in tiredness and dyspnea from the previous treatment period to the next (Patients 1–7 had RHD)*

The signs indicate improvement, + or impairment, − which was very slight and somewhat uncertain, + slight but unmistakable, + + moderate, + + + or considerable, + + + +

Period Pat. no.	I Digoxin dose		II 0.125		III 0.25		IV 0.375		V 0.50		VI 0.75		VII 1.00		Sinus rhythm
1	0	333	+ + +	400	+ +	433	+ +	417	+ +	450					+ + + +
2	.375	750		700[a]	+ + +	700	0	725	+ +	725					
3	.375	650		475[a]	+ + + +	575	+ + +	625	+ +	700	+	700			0
4	.375	825	− −	750[a]	+ +	775	+ +	775	+	800	0	800		650	
5	.25	475	−	450	+ +	475	+	475	0	500					
6	.375	383		400	+ + +	383	+ +	417							
7	.375	400	0	400	+ +	400	+ +	400	0	417					
8	.50	600	−	525[a]	+	575	+ + +	600	+	650	+	725			
9	.375	400	0	450	−	467	+	517	+ +	533	−	533[b]			
10	375	417	0	433	+ +	450	0	450	+	500	0	483	0	500	+ +
11	.375	675		675[a]	+ + +	750	0	725		675					
12	375	925	0	925	0	925	0	900							
13	0	450	+	500	+ +	525	+ +	525	+	550	+ + +	625	+	600	
14	0	750	0	750	+ +	775	+	775	+	775	+	775			+ + +
Mean				560		566		595		606					
n				14		14		14		12		7		3	4

[a] More tired after this test than after the other tests.
[b] Maintenance dose 0.625 mg per day

For estimation of the digoxin concentration in plasma a method was used based on the ability of digitalis glycosides to inhibit the uptake of ^{86}Rb in erythrocytes. The method was first published by Lowenstein (33) and later modified by Bertler and Redfors (6).

Conventional statistical methods were used. Significance regarding clinical grade, digoxin tolerance changes in subjective symptoms and their correlation to other parameters were tested by non-parametric methods: the rank-sum test and the test for pair differences of Wilcoxon and the rank correlation test of Spearman. Significances regarding other parameters were tested by t-tests or analysis of variance

Levels of significance: $p \leq 0.001$ highly significant, $0.001 < p \leq 0.01$ significant, $0.01 < p \leq 0.05$ probably significant.

RESULTS

Subjective symptoms

The physical activity and the symptoms at the beginning of the investigation are given in Table II. The main complaints were *dyspnea and tiredness*. The functional state presented in the same table shows the degree of exertion which provoked the symptoms. Patients without RHD seemed mostly to be in a better condition than the patients with RHD

Also during the investigation tiredness and dyspnea at exertion were the principal symptoms. With few exceptions these complaints run parallel and are therefore discussed as an entity The changes from one treatment period to the next are presented in Table IV and Fig. 2. Only one of the three earlier undigitalized patients felt definitely better at the first digoxin dose, 0.125 mg/day At this dose most of the other 11 patients experienced a considerable increase of dyspnea and tiredness ($p = 0.02$). Most affected were those with RHD one of whom (no. 6) also had an aggravated hacking cough and orthopnea at night. Four patients felt no change of tiredness and dyspnea. Two of them (nos. 7 and 12) had at the first examination had toxic symptoms (Table V) which disappeared when the digoxin dose was lowered.

With increasing doses the dyspnea and tiredness at exertion gradually diminished (Table IV and Fig. 2 *a*) This improvement was significant already at a dose of 0.25 mg digoxin per day ($p < 0.01$). Only three patients without RHD were not clearly improved at this dose. One of them, patient 8, with ASHD and a big heart, showed no noticeable improvement until he received a higher dose. A further increase of the digoxin dose to 0.375 and 0.50 mg, respectively produced in the whole group an improvement, which at the 0.375 mg period was significant ($p < 0.01$) At the doses

which gave toxic symptoms two patients experienced clearly increased tiredness and dyspnoea, whereas three were further unmistakably improved as regards these symptoms. The dose–response curves with the doses expressed as percentage of the toxic doses are shown in Fig. 2 *b*

After the end of the dose–response study 4 patients were successfully converted to sinus rhythm with a DC defibrillator Three of the patients were further improved at sinus rhythm (Table IV)

At the beginning of the investigation only the patient with hyperthyroidism, still undigitalized, had *effort angina*. This complaint disappeared already when 0 125 mg digoxin per day was given.

When the digoxin dose was reduced to 0 125 mg daily only four patients with RHD noticed increased *palpitations*. In the following treatment periods most patients felt a slowing of the heart rate, especially those with RHD at the dose of 0.25 mg/day The feeling of rapid, uneven heart action produced, however very little discomfort. In the control group two patients (nos. 15 and 17)

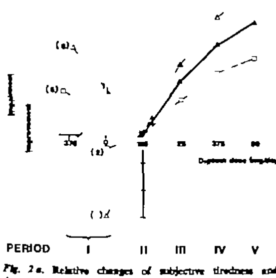


Fig. 2 a. Relative changes of subjective tiredness and dyspnea between 14-day periods in which the patients were treated with varying digoxin doses. One unit of the Y-axis means very slight improvement ↑ or impairment ↓ 2 units slight but unmistakable, 3 units moderate and 4 units considerable change of the subjective well being (cf Table IV) The zero point is set at the first dose common to all patients. Figures in parentheses denote no. of pat.

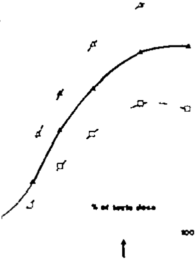


Fig. 2 b Corresponding dose–response curves showing improvement in tiredness and dyspnoea with increasing digoxin doses, 0.125 to 1 00 mg d. The doses are expressed as percentage of the toxic doses. ▲—▲ mean of all patients; △– –△ patients with RHD· □– –□, patients without RHD· patients undigitalized at the beginning of the study

felt tachycardia at the third and second period respectively

At the end of the investigation an individual *optimal"* maintenance digoxin *dose* was chosen, taking into consideration the subjective well-being, the physical working capacity and the toxic limit of the patients (Tables IV and V). The optimal" dose was on the average 0 44 mg/day (range 0.25–0 75 mg), i.e. about 65 % of the mean toxic dose, which was 0.65 mg/day The optimal" and toxic doses were somewhat lower for the patients with RHD, 0.39 and 0.59 mg respectively than for the other patients, 0.48 and 0.71 mg/day The differences were not significant. The *toxi symptoms* were mostly gastro-intestinal (Table V) and disappeared rapidly when the digoxin dose was reduced. ECG signs suggesting digitalis intoxication were noticeably few and are reported in a separate paper in which the relation between digoxin dose and ventricular rate is presented (44).

It was intended to study the control patients at unchanged, non-toxic digoxin doses. However in the last few days of period IV two of them (nos.

Table V *The maintenance dose of digoxin (mg/day) at onset of period I at "optimal" treatment and at appearance of toxic manifestations*

Pat no	Maintenance dose at onset	"Optimal" dose	Toxic dose	Toxic symptoms
1	0	0.375	0.50	Nausea
2	0.375	0.375	0.50	Nausea, anorexia
3	0.375	0.50	0.75	Anorexia
4	0.375	0.50	1.00	Tiredness, lassitude nausea, vomiting
5	0.25	0.375	0.50	Nausea, lassitude
6	0.375	0.25	0.375	Nausea, abdominal pain
7	0.375	0.375	0.50 *b.*	Tiredness, headache. *b.* Nausea
8	0.50	0.50	0.75	Nausea
9	0.375	0.50	0.625	Nausea
10	0.375	0.50	1.00	Nausea
11	0.375	0.375	0.50	Increasing dyspnea, tiredness, lassitude, nausea, vomiting
12	0.375	0.25	0.375 *b.*	Anorexia. *b.* Anorexia, nausea, vomiting
13	0	0.75	1.00	Nausea, bdominal discomfort
14	0	0.50	0.75	Nausea, vomiting

19 and 20) felt slight, recurrent nausea 1–2 hours after administration of the daily dose, and patient 17 began to lose his morning appetite. Tiredness and dyspnea were not changed. The gastro-intestinal symptoms were aggravated when the patients afterwards received 0.50 mg digoxin per day: the symptoms disappeared at once at reduced dosage. In 15 out of 18 periods the control patients felt no change of tiredness and dyspnea as compared with the first period (Table VI).

When the patients in the main group without knowing it, received for a second period the maintenance dose given in period I, seven of them found their subjective well-being to be about the same as at the beginning of the investigation, three felt better and one worse. These results, like the results in the control study support the view that the changes in subjective symptoms observed in the main study were due to changes in digoxin dosage and not to unspecific effects caused by the study itself.

Physical working capacity

The PWC estimated at exercise tests in period IV when all patients were digitalized (Table IV) was well correlated to the clinical grade assessed from the history ($p < 0.01$). At 0.125 mg digoxin per day PWC was on the average reduced by 51 kpm/min in the previously digitalized patients with RHD (Fig. 3 *a*). Among the others it was lowered only in patient 8. At this dose PWC was increased for two of the three previously undigitalized patients. It may be noted that 5 patients, particularly nos. 2 and 3 were more tired after this exercise test than after the other tests.

When later 0.25 0.375 and 0.50 mg digoxin per day was administered the PWC increased by on average, 27 ($p < 0.01$) 8 ($p > 0.05$) and 22 (12 patients $p < 0.05$) kpm/min respectively. The mean PWC was elevated from 560 kpm/min at 0.125 mg/day to 617 kpm/min at the "optimal" digoxin dose ($p < 0.01$). At the last examination, when the patients had developed toxic symptoms, PWC was on the average unchanged, i.e. very little reduced. Fig. 3 *b* shows the changes in PWC with increasing digoxin doses, when these are expressed as percentage of the toxic doses.

The patients with RHD had about 100 kpm/min lower mean PWC than the other patients. The individual variations were, however great and the difference was not statistically significant, nor were the changes of PWC from one examination to the next significantly different in the two patient groups.

When the patients were treated for a second time with the digoxin doses given in period I, the mean PWC 605 kpm/min was somewhat higher than at the first examination, 591 kpm/min. The difference was not significant. Patients without RHD showed an increased, the others mostly a slightly lowered PWC.

The PWC of the control patients (Table VI), 596 ± 164 kpm/min (mean ± S.D.), was very similar to the PWC of the patients in the main group, 595 ± 162 kpm/min when they were treated with the same digoxin dose. The mean

Table VI. *Physical working capacity (kpm/min) of the control patients at 4 exercise tests performed at intervals of 14 days. Digoxin dose 0.375 mg daily. Changes in tiredness and dyspnea from the previous treatment period to the next cf. Table IV*

Pat. no.	Periods I	II		III		IV	
15	750	0	750		750	+ +	775
16	625	0	675	0	675	0	675
17	750	−	700	+	750	0	725
18	600	0	600	0	600	0	600
19	317	−	317	+	383	0	367
20	400	0	417	0	433	0	433
Mean	574		577		599		596

PWC at the four successive examinations were 574, 577, 599 and 596 kpm/min respectively. The individuals had a S.D. between examinations of 21 kpm/min. Analysis of variance showed no significant change between examinations. These results and those from the two comparable examinations in the main group seem to indicate that no systematic and statistically significant change of PWC due to increased training or familiarity with the bicycle ergometer occurred during the progress of the examination.

Laboratory tests

At *spirometry* the VC was 87 ± 9% (mean ± S.D.) and FEV% 100 ± 6% of predicted normal values. Both VC and FEV% were somewhat lower in patients with RHD than in the others, but the differences were not significant. MVV was 83 ± 15% of predicted values and was significantly lower in patients with RHD than in the others, 71 and 95% of predicted values respectively ($p < 0.001$). The difference may reflect different functional states of the two patient groups.

ECG showed in patients 8 and 13 left axis deviation (< −30°). Otherwise no notable changes at rest were seen except for the atrial fibrillation and S–T segment depressions and changes in T configuration attributable to the digitalis treatment.

The systolic and diastolic *blood pressures* taken under basal conditions are shown in Table II. The changes during the investigation were very small. The mean values were 129/86 at the 0.125 period and 136/85 mmHg at the "optimal" dose period. The difference in systolic pressure

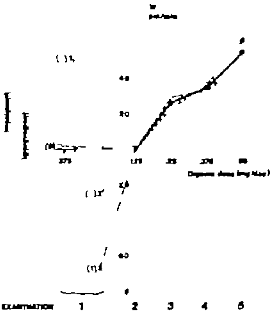


Fig. 3 a. Relative changes of physical working capacity from one exercise test to the next. The zero point is set at the first digoxin dose common to all patients. Figures in parentheses denote no. of pat.

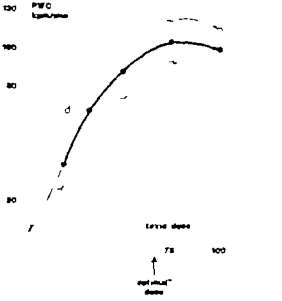


Fig. 3 b. Corresponding dose-response curves showing improvement of PWC at increasing digoxin doses, 0.125 to 1.00 mg d. The doses are expressed as percentage of the toxic doses. ●—● mean of all patients, —— patients with RHD, □— —□, patients without RHD-patients undigitalized at the beginning of the study.

Table VII *Some laboratory tests on the patients. Mean values of repeated determinations during the study given for ESR, Hb, potassium and creatinine in serum and for creatinine clearance*

Pat. no.	ESR (mm)	Hb (g/100 ml)	K/s (mEqv/l)	Mg/s (mEqv/l)	Ca/s (mEqv/l)	Creat./s (mg/100 ml)	Creat. clearance (ml/min)	BSP retention (%)
1	8	13.0	3.9	1.6	5.1	1.0	68	7
2	2	16.8	4.1	1.9	5.1	1.1	85	46
3	4	14.6	4.0	1.8	4.3	1.2	86	6
4	11	14.7	4.7	1.8	4.8	1.1	90	9
5	5	14.5	4.3	1.7	5.2	0.9	88	18
6	29	13.3	4.2	1.9	4.6	0.8	83	10
7	8	13.1	4.5	1.9	4.5	0.8	81	5
8	7	14.3	4.3	1.8	4.6	0.9	141	16
9	28	12.5	4.0	2.0	4.8	0.9	89	13
10	22	13.1	4.6	1.6	4.8	0.9	100	15
11	7	14.5	4.5	2.1	4.5	1.1	127	7
12	4	15.2	4.6	2.0	4.8	1.2	99	9
13	11	14.0	4.4	2.0	4.2	0.9	97	11
14	8	14.8	4.1	1.6	4.6	0.8	132	6
15	15	13.3	4.7	1.6	4.9	1.2	75	19
16	5	15.2	4.4	1.6	4.5	1.0	135	7
17	5	13.5	4.3	1.6	4.7	1.0	91	15
18	3	15.3	4.4	1.6	4.7	1.1	109	8
19	10	15.0	4.0	2.0		0.9	101	7
20	14	13.3	4.3	1.7	4.8	0.8	90	5

was probably significant. B.P during exercise showed a similar slight increase at higher digoxin doses.

The *heart volumes* determined by X-ray at the beginning of the investigation are presented in Table II Seven patients examined at every treatment period showed no significant changes in heart volume attributable to changes in digoxin dose.

During the investigation no significant change in *weight* occurred.

The results of some laboratory tests are shown in Table VII. The *electrolytes* were found to be within normal limits except for a few chloride values. A few patients had *PBI* and *T 3* test values outside the normal range, but both tests were abnormal only in patient 13 with hyper thyroidism. Four patients had slightly reduced *creatinine clearances* Patients with RHD had lower values, mean 83 ml/min, than the others, mean 112 ml/min ($p < 0.005$). No corresponding difference was found for *creatinine in serum*. Only patient 2 with a combined mitral valvular disease had clearly impaired *liver function tests*. Some others had a BSP retention just above normal level, but had otherwise normal liver function tests except for a slightly elevated LDH in one patient.

ESR, Hb and *serum potassium* repeatedly estimated during the investigation, remained unchanged.

DISCUSSION

Subjective well-being and its relation to the digoxin dose

Description of symptoms may be biased by the situation, the expectations and the personality of the patient (25 27) The results may also be biased by the clinician as an engaged observer Experiments with placebo illustrate the problems (5) In the present study the investigator gained an extensive knowledge of every patient and the above-mentioned difficulties were carefully taken into consideration, e.g. the size of the dose changes was minimized and unknown to the patients.

The lowest digoxin dose administered in the main group 0.125 mg daily was for most patients evidently insufficient. Only one of the previously undigitalized patients was clearly improved. Significant recovery was seen at the following dose elevations. The effect was pronounced already at 0.25 mg daily which for most patients was a rather low and not optimal" dose. This confirms the results of Weissler et al. (57), who showed that even rather small digitalis doses

produced hemodynamic effects as measured by the decrease of left ventricular ejection time. The current result may also explain the statement of Chung (13) that more than 80% of his patients "had done well with 0.25 mg digoxin by mouth daily". In the present study, however, it was shown that tiredness and dyspnea at exertion were further improved with increasing digoxin doses (Fig. 2 *b*), in some patients up to a level close to the toxic dose. Patients with and without RHD showed a somewhat different reaction. The former deteriorated more at reduced digoxin dose and showed a more pronounced improvement when the dose was later raised.

The mean "optimal" maintenance dose, 0.44 mg daily, was about 65% of the toxic dose, which is in good agreement with the therapeutic toxic ratio (63%) at digitalization stated by Lown and Levine (34). Because of the great individual differences in reactivity to digitalis it seems, however, not to be always advisable to look for an "optimal" dose defined as a certain per cent of the toxic dose. In patients who have little effect of the digitalis treatment (no. 10) a dose with a wide margin of safety to the toxic limit can be recommended. In other patients the "optimal" dose may lie very close to the toxic dose, as illustrated by some patients with RHD (nos. 1, 2 and 6). Estimation of the digoxin concentration in plasma may be of assistance when judging the danger from toxicity to the patients (7, 46, 53).

There is a confusing variation in the average digoxin maintenance doses recommended in the literature. Thus Gold et al. (19) advocated 0.75 mg/day as the most satisfactory dose, seldom causing toxic symptoms, whereas Chung (13) recently found 0.25 mg daily to be an adequate dosage for most patients. In the present study a mean "optimal" dose of 0.44 mg was found. These variations may have several explanations, such as different criteria of adequate therapeutic effects, different composition of the patient materials, and altered sensitivity due to the varying use of diuretics. Such factors, however, can hardly explain entirely the divergent recommendations. Different biopharmaceutical properties of the digoxin preparations administered by different investigators have probably had an effect as well (19).

A method of predicting the "optimal" digitalis dose or the toxic limit of a patient would imply a great progress in digitalis treatment, and the possibility to do so from clinical findings was examined in this study. Such a prediction was, however, not possible from the subjective improvement seen at different digoxin doses because of the great individual variations. A good improvement on a low dose did not mean that the "optimal" dose had been reached. A lack of effect indicated in some patients a need of higher doses, whereas in others no greater benefit was obtained by increasing the dose. The prediction problem was in this patient material studied also in other ways (44). Estimation of digoxin concentration in plasma has given promising results. It may be possible to predict the toxic limit of a patient from plasma concentrations estimated at subtoxic dose levels (46).

Physical working capacity and its relation to subjective symptoms at different digoxin doses

In clinical routine one often has to evaluate exercise tolerance from sub-maximal exercise tests with the patients' subjective symptoms as limiting factor. It has been shown that the PWC, estimated in this way at a "very laborious" work intensity, was fairly well related to the maximal working capacity in healthy subjects (9, 10). In the present investigation the patients co-operated very well and were, with a few exceptions, made to work to the same degree of subjective tiredness and dyspnea at every test. The possibility of obtaining comparable results from time to time was further increased by the strictly standardized examinations, the small work load increments, the hard exertion with rapidly increasing tiredness and dyspnea at the highest work load.

With increasing doses of digoxin PWC was improved up to the "optimal" dose level (Fig. 3 *b*). Sub-optimal digoxin doses may thus have a rather good effect, but attempts to find individual "optimal" doses may further improve the result of the treatment.

A highly significant correlation was found between the changes of subjective symptoms and PWC when all the digoxin doses were simultaneously taken into consideration. In individual cases and at single dose steps the correlation was not so good. The mean improvement of PWC from period II (digoxin dose 0.125 mg daily) to the period with the "optimal" dose was re-

Table VII. *Some laboratory tests on the patients Mean values of repeated determinations during the study given for ESR, Hb, potassium and creatinine in serum and for creatinine clearance*

Pat. no	ESR (mm)	Hb (g/100 ml)	K/s (mEqv/l)	Mg/s (mEqv/l)	Ca/s (mEqv/l)	Creat./s (mg/100 ml)	Creat. clearance (ml/min)	BSP retention ( )
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9	28	12.5	4.0	2.0	4.8	0.9	89	13
10	22	13.1	4.6	1.6	4.8	0.9	100	15
11	7	14.5	4.5	2.1	4.5	1.1	127	7
12	4	15.2	4.6	2.0	4.8	1.2	99	9
13	11	14.0	4.4	2.0	4.2	0.9	97	11
14	8	14.8	4.1	1.6	4.6	0.8	132	6
15	15	13.3	4.7	1.6	4.9	1.2	75	19
16	5	15.2	4.4	1.6	4.5	1.0	135	7
17	5	13.5	4.3	1.6	4.7	1.0	91	15
18	3	15.3	4.4	1.6	4.7	1.1	109	8
19	10	15.0	4.0	2.0		0.9	101	7
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Digitalis intoxication may appear at any dosage (48). In the current investigation toxic symptoms or signs developed at doses from 0.375 to 1.00 mg. All patients, though treated with a purified glycoside developed nausea, and some vomited. Loss of appetite, often said to be the earliest manifestation of digitalis intoxication (13) was seen in only 3 patients. The symptoms usually developed about 1 hour after the digoxin dose was administered, concurrent with the plasma peak level of the glycoside (46) and disappeared rapidly when the dose was reduced. The ECG signs of digitalis intoxication were few (44)

This old picture with dominating gastrointestinal disturbances and few ECG signs of intoxication may be due, inter alia to the early detection of toxicity resulting in a proportionately low myocardial concentration of the drug. The absence of interacting drugs disturbing the electrolyte balance and the sensitivity of the patients probably also played a rôle

Aggravation of pre-existing congestive heart failure may be an effect of digitalis intoxication (1.., 32, 49), and is considered mostly to be due to cardiac arrhythmias. Perhaps an impaired myocardial function caused by other toxic effects than an arrhythmia may be present as well (13). In the present study this was suspected in patient 11 who developed increased dyspnoea at the toxic dose. He showed with increasing digoxin dose an increasing number of ventricular aberrations at exercise but no arrhythmia attributable to the digoxin treatment. Lassitude and increasing tiredness were also observed and, like the aggravation of congestive heart failure, are important possible toxic manifestations of digitalis to note.

ACKNOWLEDGEMENTS

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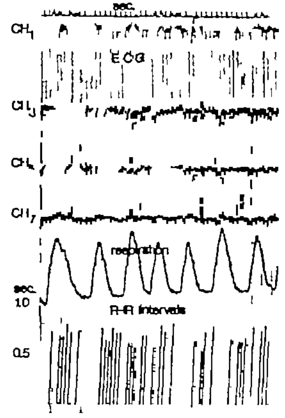


Fig. 1. Recordings registered at all examinations (from above): time (sec); ECG; respiratory curve and lengths of the R–R intervals (sec).

size of the doses administered. At the beginning of the study three of the patients were undigitalized, the others had been given an unchanged digoxin dosage for at least 14 days, mostly 0.375 mg/day.

The control patients were studied in order to evaluate the possible unspecific affects caused by the study itself, i.e. increasing familiarity with the procedure and possible training effects. The controls, too, were given specially prepared tablets (Draco, Lund, Sweden), all of which, however, contained 0.25 mg digoxin. Receiving 1 / tablets daily of the different looking preparations the controls, without knowing it, continued with their previous maintenance doses during periods II–IV (30).

On the last day of each treatment period all patients were carefully examined by the author. A thorough history was taken, electrocardiogram and ventricular rate at rest and during exercise were registered and blood samples drawn for estimation of, inter alia, plasma digoxin concentration and serum potassium.

Electrocardiogram. At rest 1-lead ECG was recorded on a six-channel direct writing ECG apparatus, Mingograf 81 (Elema-Schönander, Sweden). During exercise leads CH were continuously registered.

Exercise tolerance tests were performed on an electrically braked bicycle ergometer (Elema-Schönander, Sweden) with the patients in sitting position. The work load was increased every 6th min without pauses to rest between the loads. The following work loads were used: for men 150, 300, 450 etc. kpm/min and for women 100, 200, 300 etc. kpm/min. The patients were made to work until they could not continue because of tiredness and dyspnea (30).

Physical working capacity (PWC) was estimated in these exercise tests as previously described (30).

Ventricular rate at rest, during and after exercise was continuously measured with an electronic cardio-tachygraph. This was specially constructed for the investigation and was connected to the Mingograf 81 which registered the R–R intervals as vertical lines, the lengths of which were proportional to the intervals (Fig. 1). A system of relays simultaneously registered and arranged the R–R intervals according to their lengths into classes with width of 50 msec. After supine rest for at least 30 min mean ventricular rate and the lengths of the R–R intervals were registered during 4 min of rest. Evaluation of the ventricular rate during exercise from one-minute periods will in patients with atrial fibrillation be subject to random errors due to the lability of the ventricular rate. Counting ventricular rate from more than one minute will, on the other hand, increase systematic error associated with the steady state problem. In order to balance these errors, the "steady state" ventricular rate during exercise given in this presentation was calculated from the ventricular rate of the last three minutes of each work load. According to preliminary study (33) the following formula was used: $(VR_4+2VR_5+3VR_6)/6$, where VR_i is the ventricular rate from minute i.

RESULTS

After treatment with 0.125 mg digoxin daily for 14 days the mean *ventricular rate at rest* was reduced by 12 beats/min in the three previously undigitalized patients (Fig. 2). At this dose the mean rate of all 14 patients in the main study was 78/min. When the digoxin dosage was elevated to 0.25, 0.375 and 0.50 mg daily the mean ventricular rate successively slowed down to 69, 65 and 60 beats/min. Seven patients, who tolerated higher doses, showed a further significant reduction of ventricular rate at the following dosage.

The individual differences in ventricular rate were great—in the 0.125 mg period the rate varied from 52 to 101/min. The scattering was less pronounced at high doses, but was still noticeable. Some patients had a higher rate at toxicity than others at quite insufficient digoxin doses. Patients with rheumatic valvular heart disease (RHD) had a lower mean ventricular rate than patients without RHD ($p<0.05$ for doses up to 0.50 mg/day). The decrease of ventricular

rate from one dose to the next was, however not significantly different in the two patient groups.

At the end of the investigation an "optimal" digoxin dose was chosen for each patient (Table I), taking subjective well-being, physical working capacity and the toxic limit of the patient into consideration (30). At the mean "optimal" dose, 0.44 mg/day the mean ventricular rate was 61/min (range 47 to 75/min, Table I, Fig. 2). The ventricular rate at the dose most suitable to control symptoms thus differed considerably between patients. Patient 3 exemplified the difficulties in clinical management of digitalis therapy. He had at 0.25 mg digoxin/day a low ventricular rate of 50/min. Despite this the dose was increased and he further improved both at 0.375 and 0.50 mg/day (30) without any change in the ventricular rate at rest. The last dosage (0.50 mg/day) was considered as "optimal" for the patient. The mean "optimal" dose was somewhat, although not significantly lower for the patients with RHD than for the others (30). No significant difference in mean ventricular rate at the "optimal" dosage, 60 and 63/min respectively was found between the two patient groups.

At the dose which caused toxic symptoms, on the average 0.65 mg/day the mean rate at rest was 56/min, varying from 39 to 66 beats/min.

The possibility of predicting the "optimal" dosage or the toxic limit for a patient from ventricular rate measured at a lower non-toxic dosage was tested. Ventricular rate at 0.375 mg

Table I. *"Optimal" digoxin doses and ventricular rate at rest and at low, medium and high work loads at this dosage*

Pat. no	"Optimal" dose (mg/day)	Ventricular rate at "optimal" dose: Rest	Exercise: Low	Medium	High
1	0.375	69	112	152	175
2	0.375	75	97	152	171
3	0.50	49	71	86	136
4	0.50	60	74	97	111
5	0.375	47	72	89	138
6	0.25	58	94	143	163
7	0.375	60	82	136	177
8	0.50	71	112	132	154
9	0.50	57	84	103	124
10	0.50	70	103	147	182
11	0.375	52	79	104	136
12	0.25	59	91	163	194
13	0.75	68	95	107	149
14	0.50	64	86	113	153
Mean ± S.D.	0.44 ± 0.13	61 ± 9	89 ± 14	123 ± 26	154 ± 23

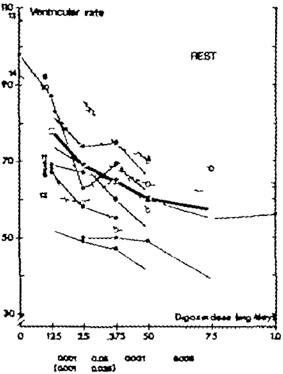


Fig. 2. Ventricular rate at rest in patients treated with increasing digoxin doses. Figures at beginning of the curves denote patient numbers, p = significance of the differences between adjacent examinations; the significances when patient 12 is excluded from the calculations are shown in brackets. ●—● patients with RHD. ○—○ patients without RHD. ⊞—⊞ mean of all patients. ⓐ or ○ = ventricular rate at "optimal" doses.

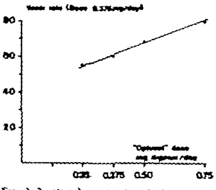


Fig. 3. Ventricular rate at rest at maintenance digoxin dose of 0.375 mg/day correlated to the "optimal" dosage of each patient. $r = 0.5$, $0.05 < p < 0.1$ (patient 12, ⊞, is excluded from the calculation).

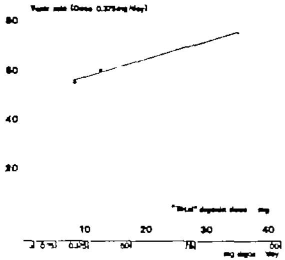


Fig 4 Ventricular rate at rest at the digoxin dosage 0 375 mg/day correlated to the total digoxin doses administered from the 0.125 mg period to appearance of toxicity The corresponding daily dosage during the different treatment periods is indicated below -0.53; $0.05 < p < 0.1$ when patient 12, [+] is excluded from the calculation.

digoxin/day was plotted against the dosages later found to be optimal" for the patients (Fig. 3). Although the patient with the highest optimal dose (0 75 mg/day) at 0.375 mg/day had a high ventricular rate the scattering of the rate was too great for patients whose optimal dosages were 0.375 or 0.50 mg/day the correlation between the rate at 0.375 mg/day and the optimal" dosages was not significant ($r = 0.55$ $0.05 < p < 0.1$) A similar low correlation was found between ventricular rate at 0.375 mg/day and the toxic limit of the patients, expressed as the total digoxin dose given from the 0.125 mg period to appearance of toxic symptoms (Fig. 4)

The effect of different digoxin doses on *ventricular rate during exercise* is shown in Fig. 5 When the digoxin dose was increased from 0 125 to 0.25 mg/day a highly significant decrease of ventricular rate was attained at the different loads. The mean reduction of the rate was not so pronounced in the next treatment period (0.375 mg/day) but this was mainly due to a divergent reaction of patient 12, especially at low work load.

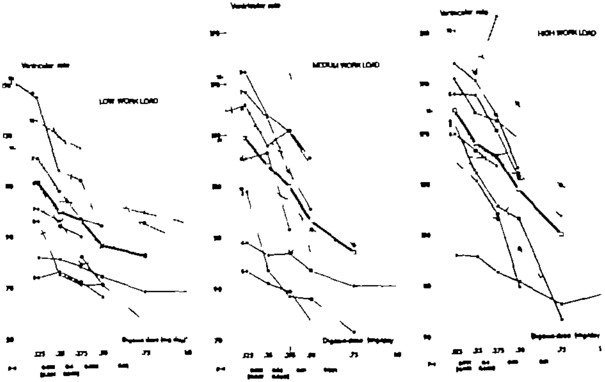


Fig 5 Ventricular rate at low medium and high work loads in patients treated with increasing digoxin doses. Symbols as in Fig. 2. Low work load = 100/150 kpm/min for women and men respectively Medium work load = 200–300/300–450 kpm/min. High work load = 300-400/450–900 kpm/min = the highest work load managed by the patient in 6 min at all the treatment periods.

At the following doses the mean ventricular rate was further significantly lowered. At all work loads great individual differences in ventricular rate were found. This was also the case at the "optimal" dosage, at which the rate varied from 71 to 112/min at low work load and from 111 to 194/min at high load (Table I). Most patients, however, retained their own characteristic rate pattern at the varying dosages. Patients with a high rate at a low digoxin dose mostly had a relatively high rate also at high digoxin doses. Also during exercise the patients without RHD had, on the average, a somewhat higher ventricular rate than the other patients. The difference between the groups was, however, mostly unsignificant.

The correlations between ventricular rate at low work load at a digoxin dose of 0.375 mg/day and the "optimal" dosage or the toxic limit were lower ($r = 0.45$ and 0.29 respectively; $p > 0.1$) than those found for ventricular rate at rest. At medium and high work loads there was no correlation at all.

The relation between the decrease of ventricular rate and the digoxin doses expressed as percentage of the toxic doses is shown in Fig. 6. At rest the rate-reducing effect of the glycoside was relatively more pronounced at low dose levels. The dose-response curves during exercise were

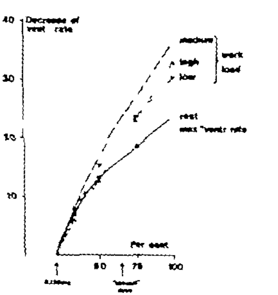


Fig. 6. Decrease of ventricular rate at rest and exercise at increasing digoxin doses expressed as percentage of the toxic doses. Mean values of 13 patients. (Patient 12 excluded.)

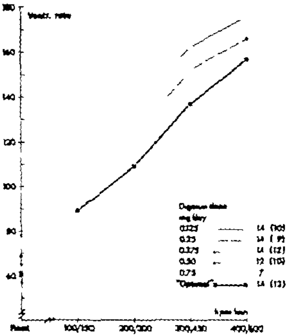


Fig. 7. Mean ventricular rate at rest and during exercise at different digoxin doses. Figures in parentheses denote the reduced number of patients at 400/600 kpm/min.

more straight. Calculated in absolute figures the digoxin effect was most pronounced at medium work loads. Relatively, however, ventricular rate decreased on the average 21% at rest when the dose was elevated from 0.125 mg to "optimal" dose levels. Corresponding figures for low, medium and high work loads were 19, 16 and 11% respectively. The highest ventricular rate registered at the different exercise tests (max. ventricular rate) was least influenced and was at "optimal" doses reduced by an average of only 6%.

Similar dose-response curves for subjective tiredness and dyspnoea and the PWC (30) plotted on arithmetic scales showed moderately bent profiles, levelling off especially above the "optimal" dose levels. The changes of ventricular rate at rest and during exercise from one treatment period to the next were well related to the corresponding changes in subjective symptoms ($p < 0.001$ at rest and medium work load, $p < 0.01$ at low and high work load). A similar correlation was found to the PWC ($p < 0.01$). In individuals and single dose steps, however, the relationship varied.

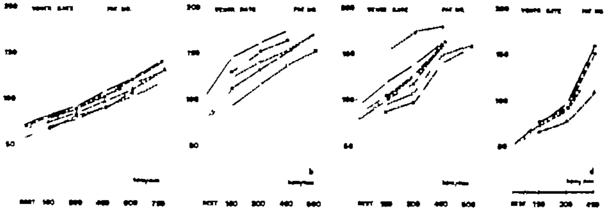


Fig 8 Ventricular rate at rest and exercise in four patients treated with increasing digoxin doses. Symbols as in Fig. 6 in addition: ★ ★ before digitalization ■ ■ 1.0 mg digoxin/day

The mean responses of the ventricular rate to exercise are shown in Fig. 7 These curves might give the false impression that ventricular rate in patients with atrial fibrillation always increases linearly with the work load It is true that a linearity could be recognized in most patients—but only at certain digoxin doses (Fig. 8) A linear increase at all the digoxin dosages was found only in patients 4 and 14 Mostly the rate increased quite non-linearly In some patients (nos.

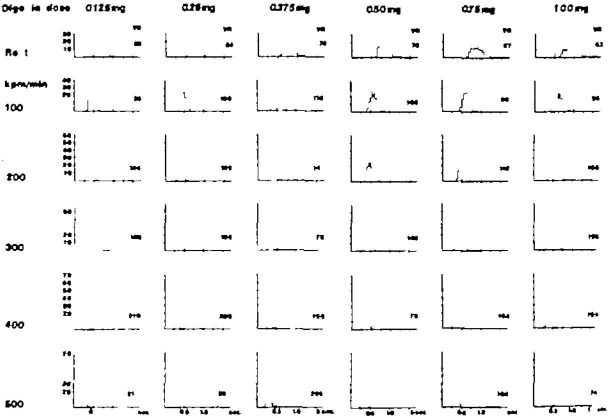


Fig 9 R R interval histograms of patient 10 treated with increasing digoxin doses The intervals are arranged according to their lengths in classes with width of 50 msec and shown in of the total number of beats. The histograms represent at rest steady state periods of 24 min, at exercise the last 3 min on each work load VR = mean ventricular rate

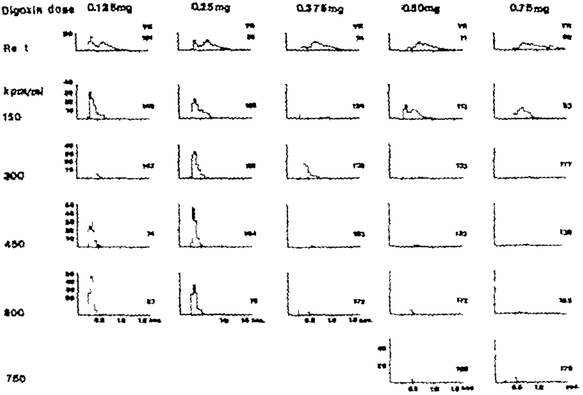


Fig. 10. R–R interval histograms of patient 8. Compare Fig. 9. Note the bimodal shape of the histograms at rest and low work load.

1, 8, 10, 12, 13) a proportionately rapid acceleration of ventricular rate from rest to the lowest work load was seen. Such an increase of ventricular rate has previously been reported (39, 4.) and is here illustrated in Fig. 8 *b* and *c* at low digoxin doses and in Fig. 11 at a toxic dose. Otherwise the rate curves at the high doses in most patients became more straight (Fig. 8 *b*): they often rose stepwise or were even concave upwards with slow increase of ventricular rate at low or medium work loads (Fig. 8 *c*). In some patients (nos. 3 and 5) an exaggerated increase of ventricular rate occurred at high work load at most doses administered (Fig. 8 *d*). Despite the different reactions of ventricular rate to exercise, patients with a high rate at rest mostly had corresponding high rates also at work.

The complex problem of the *irregularity of ventricular rhythm* in atrial fibrillation was studied in this patient material but will be discussed in greater detail separately (34). Only a few examples are given to show how digoxin treatment in different ways may modify the lengths of R–R intervals and consequently the ventricular rate. R–R interval histograms of patient 10 are presented in Fig. 9. The intervals were successively shortened at increasing work loads; at increasing digoxin doses they became longer and more scattered. The R–R intervals were gathered around a single peak except at rest at high digoxin doses. A similar interval pattern was seen in three other patients, but their intervals were more scattered and the histograms showed a more pronounced right oblique distribution. Patient 8 (Fig. 10) at the *different digoxin doses* had a ventricular rate at rest and exercise well comparable with the rate of patient 10. The R–R interval pattern was, however, quite different. At low digoxin doses, two interval peaks (modes), about 0.25 sec. apart, were seen at rest. At high doses these two modes appeared at low work load. This characteristic interval pattern with two or more repeatedly appearing interval peaks could be recognized in 10 out of the 14 patients. Bimodality appeared

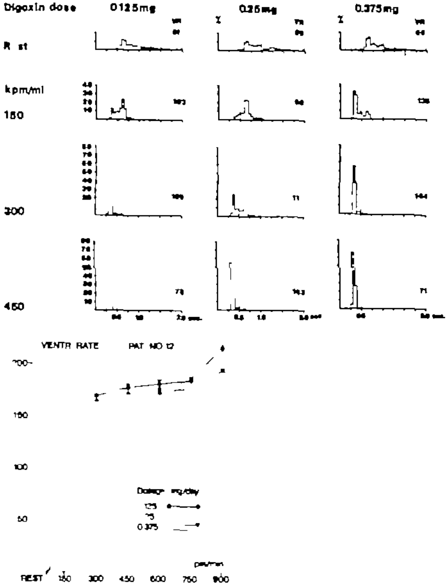


Fig. 11 R R interval histograms and ventricular rate at rest and exercise for patient 12 treated with 0.125 0 25 and 0 375 mg digoxin day Note the rapid elevation of the rate at low work loads when the patient on two different occasions was given the toxic digoxin dose, 0.375 mg day

at rest or during exercise, irrespective of the digoxin dose administered, when the ventricular rate was about 90–120 min The number and the lengths of the dominating R–R intervals in the modes at rest and during exercise were influenced by the digitalis dosage, but the differences between the modes, mostly about 0.25 sec, were fairly unchanged. A rapid shift of the intervals between two modes, sometimes seen during exercise, resulted" in a rapidly exaggerated increase of the ventricular rate (patients 1 3 5 and 1 Fig. 11)

Digoxin dosage was elevated in all patients until *manifestations of digitalis intoxication* appeared. At toxicity three patients had a ventricular rate at rest of 50/min or less. Despite methods well adapted to show typical digitalis-induced arrhythmias such as A–V junctional tachy cardia with or without exit block (22, 38), no such episode was recognized. Prolonged R–R

intervals were at rest frequently present at high digoxin doses but were also seen at low dosage in all patients but one. Anomalous beats, i.e. ventricular complexes with a configuration different from the dominant pattern of conducted beats, were registered in 13 out of the 14 patients. Classification in aberrant or ectopic beats was made according to the criteria of Marriot and Sandler (26). In some patients the distinction was difficult and in two patients no classification could be made. Aberrant beats with a RBBB configuration seemed to be most common and were diagnosed in 9 patients. The aberration appeared at all dosages, increasing with the dose in only two patients. Aberrant beats were more frequent during exercise than at rest. Ectopic ventricular beats were recognized in two patients and were as common at rest as during exercise, occurring at different dose levels.

A rare, probably toxic reaction was seen in patient 12, who also in other respects showed a divergent reaction to the glycoside (30, 32). At the digoxin dosage which gave toxic gastrointestinal symptoms, he showed an exaggerated acceleration of ventricular rate at low work loads despite a high PWC (Fig. 11). This reaction at toxicity was repeatedly reproduced. A rapid shortening of the R–R intervals from one mode to another was registered in the interval histograms (Fig. 11). This patient showed no other ECG signs of toxicity. The toxicological problems in this study are further discussed elsewhere (30, 32).

When 11 of the patients were again examined after treatment with the same digoxin doses as at the first examination, mean ventricular rate was slightly reduced. At rest it was 3 and at low medium and high work loads 9, 5 and 1 beats/min lower respectively than at the first examination. These differences between examinations performed about 6 weeks apart were not significant. Mean ventricular rates in the *control patients*, who unknown to themselves, in four periods were treated with an unchanged maintenance dose of 0.375 mg digoxin/day are shown in Table II. A slight but only for the medium work load probably significant decrease of the rate occurred during the study according to analyses of variance. In the last few days of period IV slight gastrointestinal symptoms appeared in three of the controls, probably indicating the development of digitalis intoxication (30). Simultaneously a fairly

Table II. *Ventricular rate at rest and exercise (mean ±S.D.) of the control patients*

	Examination periods			
	I	II	III	IV
Rest	71±12	68±11	68±9	64±9
Work load				
Low	100±10	96±16	93±9	88±9
Medium	132±23	126±23	125±22	119±23
High	153±28	146±25	148±22	143±27

high digoxin concentration in plasma (1.5 ng/ml) was registered in two of them. These findings fit well together indicating an unexpected non-steady state condition. The results in the control study combined with the results from the comparison between the two equivalent examinations in the main study are, however, regarded as support for the view that the study in itself did not appreciably influence the results in the main dose-response study.

DISCUSSION

The decrease of ventricular rat

The relation between digoxin dosage and ventricular rate at rest in patients with atrial fibrillation has previously been studied only by Gold et al. (1) in a way comparable to the present investigation. The relative changes in ventricular rate between different digoxin doses in the present study were in good agreement with their results, being somewhat more pronounced at low than at high dose levels. In the present study a special rate pattern previously described by Gold et al. (13) was also observed. the ventricular rate was considerably reduced at small digoxin doses, but then remained unchanged despite increasing doses until it was further reduced at high doses. Gold et al. (14) thought that this was due to a vagal digoxin effect at low doses followed by extravagal influences of the glycoside at high doses. In the present study only one patient (no. 3), however, showed such a plateau in the effect-dosage curve, and contrary to the findings of Gold et al. this patient did not experience full therapeutic effects from the dosage which first gave low ventricular rate.

In 19 4 Blumgart (3) gave the first report on the reaction to exercise of the heart affected

by auricular fibrillation He concluded that digitalis in therapeutic doses failed to protect the ventricles from exaggerated response to exercise. Since then ventricular rate during exercise in patients with atrial fibrillation has been studied in many investigations (10 16, 17 19 23 36, 39 4., 43) With few exceptions (27 41) however the main intention has not been to study the relationship between ventricular rate and digitalis treatment thus the digoxin doses are not given in detail and our knowledge of the extent to which digitalis may influence the response of ventricular rate to exercise is still insufficient. Surprisingly the present investigation seems to be the first in which the effect of different digoxin doses on ventricular rate during exercise in patients with atrial fibrillation has been studied in greater detail

For the sake of simplicity the ventricular rate at rest and during exercise has, throughout the presentation of this study been plotted against the digoxin dosage on arithmetic scales. Admittedly semilogarithmic dose-effect curves might have been more appropriate, giving straight ventricular rate curves at rest and at low work loads. However a logarithmic presentation did not provide straight relationships between dose and ventricular rate in all patients, particularly not at high work loads, where a more straight decrease of ventricular rate was seen on a linear scale.

Increasing doses of digoxin were, in accordance with previous knowledge (23 27 39 41), found to modify the ventricular response to exercise. The effect of the drug was most pronounced at medium and low work loads, i.e. at strain levels most common in daily life. The rate at nearly maximal work loads was least influenced. This may be of practical importance and may have contributed to the discrepancy found in this study (30) between the subjective improvement at increasing digoxin doses and the less pronounced improvement of PWC registered in exercise tests during corresponding treatment periods.

The relation of ventricular rate to subjective symptoms and PWC

In most patients there was a fairly close relation between the rate-reducing effect of digoxin and the effect of the drug on subjective symptoms and PWC. Great individual differences in the reactions were, however seen. In some patients (nos. 9 and 10) digoxin had a pronounced negative chronotropic effect, whereas the subjective well-being was very little influenced. In others (i.e. nos. 1 4 6) the subjective improvement was pronounced, despite limited changes of ventricular rate. Patients with RHD seemed to be somewhat more improved subjectively by an increased digoxin dosage than the others (30) A corresponding difference regarding ventricular rate at rest or exercise was not found between the two patient groups. Most patients with RHD had mitral stenosis and were probably haemodynamically more assisted by the decrease of ventricular rate (42) even if the slowing was moderate. One patient with hyperthyroidism experienced a good subjective effect of the digoxin treatment and showed considerably reduced ventricular rate. His optimal dose was, however high, 0 75 mg/day

Great individual differences in ventricular rate at all digoxin doses were seen at exercise as well as at rest. The scattering was great also at the "optimal" digoxin dosage (Figs. 2 and 4) Obviously a low ventricular rate at rest or exercise is not a reliable index of adequate digitalis therapy Consequently it was impossible to decide from the ventricular rate whether a dosage (i.e. 0.375 mg/day Fig. 3) was too high, optimal or too low As other reliable methods for judging the adequacy of digitalis treatment are not available at present, new methods are necessary (25) if the frequent digitalis intoxications (8 11 20 21) are to be avoided Estimation of digoxin in plasma may be such a method. In the present study promising results concerning the prediction problem were obtained (32, 35). The correlation between plasma digoxin concentration estimated at a dose of 0 375 mg digoxin/day and the "optimal" dosage or the toxic limit of the patients was high ($r = -0.91$ and -0.89 respectively $p < 0.001$). (Patient 12 showed also in this respect a divergent reaction and was excluded from the above mentioned figures.) If these results hold also for patients in the clinical routine, it may be possible in individual patients to predict adequate digoxin dosages from blood samples drawn at subtoxic dose levels.

Even if its prediction value is low estimation of ventricular rate in patients with atrial fibrillation may still be of some value in the clinical routine because of its simplicity The rates re

gistered at rest and at low work loads seem, as isolated measurements, to have about the same value and supplement each other

The increase of ventricular rate during exercise

Ventricular rate during exercise in patients with atrial fibrillation is said by some authors to increase linearly (17 36). This is denied by others (10, 39 42) The importance of the digoxin dosage in this connection has not been adequately recognized before. In the present study the small work load increments during exercise facilitated study of the relation between ventricular rate and exercise loads. The successive changes of the profiles of the rate curves from one dose to another showed that they did not arise by chance. Digoxin dosage as well as individual characteristics influenced the increase of ventricular rate at increasing work loads. The controversial statements in the literature about the linearity are probably explained by the discrepancy between a fairly linear increase of mean ventricular rate (Fig. 7) and the different profiles of individual rate curves. Differences in the patient materials studied and the digitalis treatment given may also contribute to the divided opinions. Great errors *may however* be introduced if such mean values are used as a basis for evaluating, for instance, physical working capacity at certain ventricular rates in individual patients (36). The configuration of the individual ventricular rate curves was apparently not related to other clinical findings such as aetiology of the atrial fibrillation, clinical grades and PWC.

At optimal dosage the ventricular rate, with only a few exceptions (patients 1 and 8), increased fairly linearly or with upward concave rate curves. A patient with an exaggerated increase of ventricular rate at low work loads, forming an upward convex curve, was thus mostly inadequately digitalized (cf 39 42). An exercise test with small work load increments may therefore be valuabl in the evaluation of the adequacy of digitalis treatment. Unfortunately however straight or upward concave curve is not always reliable criterion of sufficient digitalis dosage (patients 4 5 and 13), or does special shape of the curve indicate an impending digitalis intoxication. This may sometimes be present even when an exaggerated increase of ventricular rate at low work loads is seen (42)

The R–R intervals

In 1950 Söderström (37) showed that R–R intervals of certain lengths often dominated the apparently irregular ventricular response in atrial fibrillation. This was especially demonstrable when ventricular rate was slowed after exercise. The multimodal interval pattern has been established by others (1 15 18) and is thought to be due to concealed conduction in the A–V junction and to simultaneous changes of the refractory period of the junction (9 24 28 29 37) Whether there is an intrinsic rhythmicity with a tendency to intervals of equal length to group together is still a matter of dispute (5 6, 7 15). Using autocorrelation technique Bootsma et al. (5) recently concluded that the effect of randomly spaced atrial impulses of random strength reaching the A–V node from random directions could explain the random nature of the ventricular rhythm.

In the present investigation in which the R–R intervals were studied at rest and at several different work loads the R–R interval histograms showed in most patients two or more different interval modes. They could often be followed from one work load to another and from one digoxin dosage to another Such recurrent interval patterns could not be of a random nature, only the distribution of the interval lengths within each mode. The bimodality during exercise, however was found only at a ventricular rate of about 90–120/min. Bootsma et al., studying patients only at one work load, may have failed to find these characteristic patterns, which probably are a prerequisite for finding a non-random rhythmicity

Ventricular rate during exercise seemed in the present study to increase in two different ways: (*a*) The R–R intervals were distributed round a single, more or less widespread interval peak and were successively shortened. This mechanism alone was in all patients responsible for the increase of ventricular rate at high rates when all the intervals were distributed in a narrow interval peak. (*b*) The intervals were stepwise shortened, resulting in bimodality of the histograms at certain rate levels. The uni- and the bimodal interval patterns could give fairly similar increase of ventricular rate during exercise. The pronounced and rapid acceleration of ventricular rate seen in some patients, usually from about 90 to 140/min, was however related to the rapid shift of majority of intervals from an interval

mode with longer to a mode with shorter interval length. Combined with the successively shortened intervals at lower and higher rates the stepwise elevated configuration of the rate curves was produced.

When a bimodality with one mode consisting of short intervals was present at rest, ventricular rate often showed an exaggerated acceleration during exercise. Otherwise it was not possible to predict the increase of the rate at exercise from the R–R interval pattern found at rest. These patterns did not seem to be related to other clinical parameters such as the aetiology of the atrial fibrillation, the heart volume, the PWC or the digoxin tolerance The problems concerning the R–R intervals will be further discussed separately (34).

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TIME COURSE FOR THE CHANGE IN HEMOGLOBIN CONCENTRATION WITH CHANGE IN POSTURE

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Abstract. Hemoglobin concentration has been determined at short intervals in eighteen healthy volunteers hen changing from upright to recumbent position. The average maximal decrease, 5% of the initial hemoglobin concentration, was completed in 20 min.

It has been known for long time that the plasma volume, hemoglobin concentration, hematocrit value and plasma protein concentration are greatly influenced by changes in body position. Bohme (2) found that the protein concentration of serum diminished from 8.1 to 7.1 g/100 ml after 40 min recumbency. Thompson et al. (14) report an average loss of 11% of the circulating total plasma volume after standing still for 20 to 30 min. Aull et al. (1) confirmed the increase of serum protein concentration and found an unchanged percentage distribution of the serum protein fractions.

It has also been demonstrated by a number of authors (4, 6, 8, 10, 12) that physical exercise diminishes the plasma volume in the range of 7%. These well-known effects of posture and exercise are not seldom overlooked in clinical work in the determination of Hb concentration and hematocrit and other variables which follow the protein fraction of the blood. For instance normal values for Hb concentration are often estimated in sitting position (13) but generally the experimental conditions are not mentioned (7).

The purpose of this investigation was to study rate and magnitude of the change in Hb concentration associated with an alteration of posture.

MATERIAL AND METHODS

Eighteen healthy volunteers, nine men and nine women, 25–40 years of age, are studied. Hb concentration was determined in duplicate spectrophotometrically as oxyhemoglobin standardized against the cyanmethemoglobin method.

Procedure

Blood samples, 1.5 ml for each determination, ere drawn without stasis from catheter introduced percutaneously into cubital vein. The first determination (initial value) was made after the subject had been standing upright and still for 10 min and had been in upright or sitting position for at least 1 hour. The subject then assumed the recumbent position, and blood samples were drawn after 2, 5, 10 and 20 min. In nine subjects determinations were made also after 40 and 60 min.

RESULTS

Values in the text are given as mean ±1 S.E.

The mean initial Hb concentration after 10 min in the upright position was 13.8 + 0.3 g/100 ml (range 10.6–15.8). After transition to the recumbent position there was rapid decrease of the Hb concentration (Fig. 1) significant after 10 min ($p < 0.001$). The change was completed within 20 min, thereafter no further decrease was seen. The average maximal decrease was 5.0 ± 1.4% of the initial value.

There was no difference in percentage change between men and women, nor any correlation between initial Hb concentration or body length and magnitude of change (= −0.14 and 0.11).

DISCUSSION

That posture influences the concentration of different substances of the blood has been demonstrated earlier (3, 14, 15), but the time relations do not seem to have been in estigated in detail.

The present study shows that the change is fairly

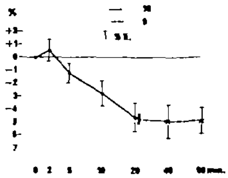


Fig. 1 Average change in Hb concentration on transition from the upright to the recumbent position % of initial value.

rapid, reaching a steady level after 20 min, an important fact to be aware of in clinical work. The observed magnitude and rapidity of the Hb change, however apply to normal individuals. The magnitude and the time course might differ considerably in patients, for instance in pregnant patients and especially in patients with disturbance of the circulation with or without edema (5 9) Thus a constant correction factor could not be used

The mechanisms behind the concentration changes are thoroughly discussed in the literature and will not be repeated here. However standardization of posture when sampling blood for determination of substances in plasma, which follow the protein fraction, is certainly needed.

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SERUM IRON TOTAL IRON BINDING CAPACITY AND TRANSFERRIN FOLLOWING ACUTE MYOCARDIAL INFARCTION

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Abstract Iron, total iron-binding capacity (TIBC) and transferrin in serum have been measured in group of patients with acute myocardial infarction (AMI) and in another group with myocardial ischaemia without infarction. Of the 20 patients with myocardial infarction two-thirds showed abnormally low values for serum iron on the first day. The lowest serum iron level was observed on the third day when all patients except one had serum iron below the normal range. Afterwards the serum iron level gradually increased. TIBC has been used as an indirect estimate of transferrin. On the first day TIBC varied within the normal range with few exceptions. Later there was significant fall in TIBC. On the third day TIBC decreased below the normal range in one-third of all patients, with later rise to normal levels. In contrast, normal or slightly reduced values for serum iron and TIBC ere found in the 20 patients with myocardial ischaemia ithout myocardial infarction.

The serum iron content and the total iron-binding capacity (TIBC) or the transferrin concentration of serum vary markedly in different physiological and pathological conditions (11). In some previous investigations a fall in serum iron during the acute myocardial infarction (AMI) has been found (4 5 15). In these earlier studies little attention was paid to TIBC or to the transferrin concentration as a possible factor contributing to the changes in serum iron. In the present study simultaneous determinations of serum iron, TIBC and transferrin have been made in patients with AMI, providing a better basis for interpretation of the disturbances of the iron metabolism.

MATERIAL AND METHODS

The first part of the study included 40 patients admitted to the coronary care unit, 20 of hom (13 men and 7 omen, aged 47 to 73 years), had AMI with diagnosis based on compatible history and physical signs, characteristic ECG changes, elevations of leucocyte count and ESR, and typical increase of SGOT The patients are later referred to as the *infarction group*.

The remaining 20 patients (17 men and 3 women, aged 35 to 77 years), had not the characteristic ECG changes or elevations of serum enzyme activities. These patients were diagnosed as acute myocardial ischaemia without infarction, and are referred to as the *ischaemic group*.

Serum iron and TIBC were determined in all patients on the first, second and third day after admission. Of the 20 patients in the infarction group, 11 were re-examined on the seventh and fourteenth day while 9 patients had to be omitted from the study due to complications secondary to the infarction. The 20 patients in the ischaemic group ere discharged from the hospital within the first week.

The second part of the study included 4 men (aged 56 to 77 years) all of them admitted to the coronary care unit with AMI according to the criteria mentioned above. In these patients serum iron, TIBC and transferrin were determined at daily intervals during the first eek after admission.

All patients received anticoagulant therapy None received steroids. Faeces was regularly tested for blood with negative result. In all cases the Hb content varied within the normal range.

Venous blood samples were drawn in the morning to avoid diurnal variations in the serum iron level (8). Serum iron determination was done by the method of Ramsey (16). Normal range 70–170 µg 100 ml. TIBC determination as done by modification of *Ramsey method (1)*. Normal range: 300–430 µg 100 ml. Transferrin as determined by quantitative immunological technique described by Mancini et al. (14). Normal range 220–372 µg 100 ml.

The degree of statistical significance of the differences between paired observations and between means was determined by the *t*-test.

RESULTS

The results from the first part of the study are demonstrated in Figs. 1 and 2.

On the first day after admission the mean serum iron was 59.4 µg/100 ml in the infarction group

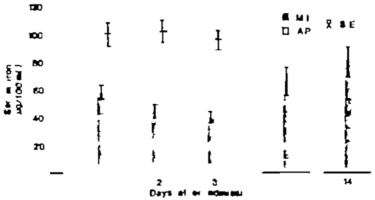


Fig. 1. Serum iron in the infarction group (dotted bars) and in the ischaemic group (open bars). Each bar represents the mean value and the standard error is represented by the vertical line through the mean.

and 100.7 $\mu g/100$ ml in the ischaemic group. The difference between these mean values was highly significant ($p < 0.001$). Fourteen of the 20 patients with infarction demonstrated abnormally low serum iron content as compared with 5 patients in the ischaemic group.

In the infarction group serum iron was approximately the same for both sexes, while in the ischaemic group the iron tended to be lower in the females.

During the first three days serum iron in patients with infarction showed a significant fall, reaching a mean value of 40.3 $\mu g/100$ ml ($0.05 > p > 0.01$). In the same period iron in the ischaemic group fell to 96.0 $\mu g/100$ ml. The reduction was not significant ($p > 0.05$). The difference in the fall of serum iron between the two groups, however, was not significant ($p > 0.05$). On the third day all but one of the patients with infarction showed abnormally low serum iron as compared with three patients in the ischaemic group.

When re-examined later during the stay in hospital the 11 patients in the infarction group showed higher values for serum iron than during the acute phase of the disease. The rise in serum iron level from the third to the fourteenth day was significant ($0.01 > p > 0.001$).

The mean TIBC on the first day after admission in patients with infarction was 321.9 $\mu g/100$ ml versus 339.3 $\mu g/100$ ml in the ischaemic group. The difference between the means was not significant ($p > 0.05$). In both groups TIBC values were approximately the same for both sexes, and varied within the normal range with a few exceptions.

During the first three days the mean TIBC in the infarction group was reduced to 289.4 $\mu g/100$ ml. The reduction was highly significant ($p < 0.001$). On the third day abnormally low values for TIBC were found in 6 out of 20 patients. In the ischaemic group the mean value for TIBC dropped to 327.6 $\mu g/100$ ml. The reduction was not significant ($p > 0.05$). Again the difference in fall of TIBC between the two groups was not significant ($p > 0.05$).

The results from the second part of the study including daily determinations of serum iron, TIBC and transferrin performed during the first week following the AMI are shown in Fig. 3.

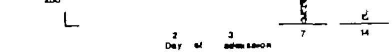


Fig. 2. TIBC in serum in the infarction group and in the ischaemic group. Symbols as in Fig. 1. Each bar represents the mean, and the standard error is represented by the vertical line through the mean.

Serum iron reached a minimum level on the third day after admission. In contrast, there was definitely a slower decline in TIBC and transferrin. These two values tended to diminish on the same scale throughout the first 5–6 days of the observation period.

DISCUSSION

Marked disturbances in serum iron, TIBC and transferrin occurred following AMI. In general, abnormally low values for serum iron were found already on the first day following the manifestation of the disease. The hypoferraemia became even more pronounced in the subsequent couple of days. Thereafter serum iron gradually was restored. In contrast, normal or subnormal TIBC and transferrin values were found on the first day. Within the first week, however, the concentrations of these two constituents gradually diminished, reaching a minimum level on the fifth to sixth day after the onset of the infarction.

Similar disturbances in serum iron, TIBC and transferrin were not detectable in patients with symptoms of myocardial ischaemia but without myocardial infarction.

As regards the variations in serum iron, the present investigation confirms the statement of previous investigators (4, 5, 15). It is evident that in the acute phase of myocardial infarction, iron is for some reason diverted from the iron-binding protein, resulting in a considerable reduction in saturation of transferrin.

Even if the reaction between iron and transferrin is dependent on pH (11), it is unlikely that the stability of the complex is influenced by the moderate variations in pH during AMI (17).

Accumulation of iron in the infarcted heart muscle may be a possible explanation of the fall in serum iron. Experiments in animals have shown that only small quantities of iron are diverted to an area of inflammation (6). It does not seem plausible that a comparatively small area such as a myocardial necrosis could take up sufficient iron to produce significant hypoferraemia.

A rapid and marked decline in serum iron has recently been described in healthy subjects during physical and emotional stress (12). Hypoferraemia has been demonstrated in animals after injection of epinephrine, adrenal cortical extracts and ACTH (3). In normal subjects the serum iron content is found to be markedly reduced after injection of cortisone (2). These results suggest that the adrenal hormones are among the regulating factors of iron metabolism. The circulatory level of noradrenaline, adrenaline and hydrocortisone are significantly elevated in the early stage of myocardial infarction, even in patients without

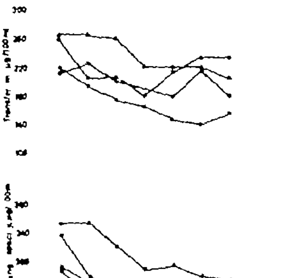


Fig. 3. Transferrin, TIBC in serum and serum iron in four men during the first 7 days following hospitalization for AMI.

obvious clinical symptoms of shock (10 13) The correlation between the fall in serum iron and an increased output of adrenal hormones in acute myocardial infarction is not clear as the rise in the latter is of much shorter duration than the more sustained hypoferraemia.

TIBC is an indirect estimate of transferrin, based on the determination of the amount of iron-reactive groups in serum with the same affinity for iron as those of transferrin It could therefore be argued that the decrease in TIBC was due to competitive substances for iron. In a limited number of patients a different method was used, based on precipitation of iron-binding protein with a specific antibody The conformity between the results indicates that the variations in TIBC represent changes in the concentrations of the iron-binding protein and not upon blocking or destruction of the iron-binding receptors of transferrin.

The significance of the variations in transferrin concentrations is not clear It seems improbable, however that the reduction was in any instances capable of impairing the iron transport, since there was an appreciable amount of unsaturated iron-binding protein still in circulation.

The mechanism regulating the transferrin level is unknown. Transferrin seems to vary closely with the albumin values (2). Diseases associated with a decreased transferrin are in general those in which the serum albumin is decreased (2, 9). This might suggest that the regulatory mechanisms of the two proteins are affected by the same agents.

The observed variations in transferrin could be due to a temporarily increased breakdown of the protein. This assumption would be in agreement with the general increase in protein catabolism seen in various acute diseases. In this connection an increased activity of the adrenal cortex is of importance, as ACTH and cortisone are known to increase the breakdown rate of albumin, while the synthesis remains normal or slightly increased (7). Thus it is possible that the hypotransferrinaemia observed in this study is another manifestation of the connection between the adrenal cortex and the iron metabolism.

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STIMULATORY EFFECT IN VITRO OF PROSTAGLANDIN E_1 ON NORADRENALINE INDUCED LIPOLYSIS IN SUBCUTANEOUS ADIPOSE TISSUE FROM HYPOTHYROID SUBJECTS

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Abstract. The lipolytic action of noradrenaline in vitro is markedly reduced in subcutaneous adipose tissue of hypothyroid subjects. This is due to an increased responsiveness of the alpha adrenergic receptors to noradrenaline, resulting in the suppression of the lipolytic effect of the hormone mediated by the beta receptor. Prostaglandin E_1 (PGE_1) inhibited noradrenaline-induced lipolysis in euthyroid subjects. In contrast, PGE_1 significantly stimulated the lipolytic action of noradrenaline in adipose tissue from hypothyroid subjects. This has been interpreted as an inhibitory effect of PGE_1 upon alpha as well as upon beta adrenergic receptor responses to catecholamines. In tissues from euthyroid subjects the main effect of PGE_1 was an inhibition of the beta adrenergic response to noradrenaline. In adipose tissue from subjects where alpha receptor responsiveness dominates, the action of PGE_1 mainly results in alpha receptor inhibition.

We demonstrated recently (14) that the in vitro lipolytic action of noradrenaline was obliterated in subcutaneous adipose tissue from hypothyroid subjects. The noradrenaline-induced lipolysis could be restored by the alpha adrenergic antagonist phentolamine. Since it is known that activation of the alpha receptor inhibits the lipolytic effect of beta adrenergic stimulation (8) it was suggested that the unresponsiveness of the hypothyroid tissue to noradrenaline was due to an enhancement of the alpha receptor response. Lack of thyroid hormone thus seems to result in a shift of the balance between the adrenergic receptors in favour of an alpha receptor response to noradrenaline.

Another group of hormones known to modulate the adrenergic receptor system is the prostaglandins (2). Prostaglandin E_1 (PGE_1) inhibited the lipolytic effect of adrenaline on adipose tissue from healthy subjects (6). The degree of inhibition, however was more pronounced when a selective beta agonist (isopropylnoradrenaline) rather than a combined alpha and beta agonist (noradrenaline) was used (7). From these studies it seemed likely that PGE_1 may attenuate both the alpha and the beta adrenergic receptor responses to catecholamines.

Since the adrenergic receptor system of adipose tissue from hypothyroid subjects is already modulated by the decreased thyroid hormone production, it was of interest to evaluate the effect of another modulator, PGE_1, on this system. The results of the present investigation show that the lipolytic response of hypothyroid adipose tissue to noradrenaline is partially restored by PGE_1.

METHODS

The clinical and laboratory findings in the 24 subjects included in this study are given in Table I. All persons had developed hypothyroidism after iodine^{131} treatment for thyrotoxicosis. The duration of the signs of hypothyroidism varied between one and half and more than six months. Nine subjects were on replacement therapy with thyroid hormone (desiccated thyroid, Thyranon® or l-thyroxin, Levaxin®). The functional state of the thyroid gland and substitution therapy was evaluated using both clinical and laboratory findings, emphasis being placed on the clinical picture rather than on isolated chemical values. In one subject, no. 7 the substitution dose was low but this could not be increased further because of palpitations. None was oversubstituted, and the high PBI values in no. 1 and no. 5 were due to iodine defilement.

Determinations of PBI, cholesterol, resin uptake of ^{125}I-labelled triiodothyronine and thyroid uptake of radioiodine at 4 hours were performed in non-fasting patients as previously published, and the same values for the normal ranges were used (14).

Subcutaneous adipose tissue (2–3 g) was obtained from the thigh of non-fasting subjects at 1 p.m. by excision under local anaesthesia, using 5–6 ml of 0.5% prilocaine-chloride, Citanest®. The fat tissue was transported in

Table I. *Clinical and laboratory findings in the subjects studied*

Pat. no.	Age (y)	Sex	PBI (μg/100 ml)	Cholesterol (mg/100 ml)	Resin uptake of T_3-I^{125} (%)	Thyroidal 24-h uptake of ^{131}I (%)	Treatment mg/d	Duration
Control group								
1	49	♀	12.7[a]	288	27	—	Thyranon 75	1 y
2	55	♀	4.2	330	28	—	Thyranon 75	4 y
3	56	♀	6.0	289	31	—	Levaxin 0.15	1.5 mo.
4	56		4.8	291	31	—	Thyranon 225	3 mo.
5	58	♀	> 30.0[a]	324	31	—	Levaxin 0.2	7 mo.
6	61	♂	3.9	317	28	—	Thyranon 75	2 mo.
7	67	♀	3.9	324	31	—	Thyranon 56	9 y
8	69	♂	5.3	264	28	—	Thyranon 113	3 y
9	71	♀	5.8	286	—	—	Thyranon 150	1 y
Hypothyroid group							Duration of hypothyroid symptoms (mo.)	
10	52		3.9	412	28	27	~6	
11	52	♀	3.2	406	24	28	~3	
12	55	♀	2.8	504	24	25	>6	
13	55	♀	2.7	441	27	21	~1	
14	56	♀	2.7	373	27	20	~3.5	
15	57	♂	2.5[b]	221	23	15	~2	
16	58	♀	4.1	338	26	14	>2	
17	59	♀	3.3	229	28	17	>3	
18	60	♀	2.9	506	23	14	>1.5	
19	64	♀	3.3	310	29	14	>1.5	
20	64	♀	4.6	420	24	16	~3	
21	65	♀	6.2	211	23	27	~6	
22	67	♀	4.1	371	24	14	>1.5	
23	70	♀	2.7	441	27	21	>2	
24	70	♀	2.2[c]	369	26	21	>6	

[a] Abnormal value due to iodine defilement.
Treatment for non-thyroid disease:
[b] Dichlorphenamide 50 mg × 3. [c] Digoxine 0.25 mg/d

0.9% NaCl and preincubated for one hour at 37°C in Krebs-Henseleit bicarbonate (KHB) buffer containing 1% bovine albumin. Separate tissue sections (50–80 mg) were then incubated for two hours at 37°C in 1.5 ml of KHB (pH 7.4) containing 3% bovine albumin and 1 mg/ml of glucose, as described earlier (14). All agents as well as basal glycerol release were tested on each subject in duplicate incubations. The production of glycerol was used as an index of lipolysis. Glycerol in the incubation medium was analysed spectrophotometrically (10, 15).

PGE_1 (supplied by Dr E. Änggård, Karolinska Institute) was dissolved in a minimum amount of absolute ethanol and diluted in 0.3 M bicarbonate buffer, pH 7.5. The maximum concentration of ethanol in the incubation fluid was 0.008 g/100 ml, a concentration which does not influence the release of glycerol in normal adipose tissue (7). In this study the effect of this ethanol concentration was tested on basal as well as on noradrenaline-induced lipolysis in hypothyroid subjects. Lipolysis was not influenced by ethanol under these conditions. (l-noradrenaline bitartrate was provided by Astra AB, Södertälje, and l-isopropylnoradrenaline-d-bitartrate dihydrate by Dr F. P. Luduena, Sterling-Winthrop Research Institute, New York, USA.)

RESULTS

The basal glycerol release in nine controls and 15 hypothyroid subjects was 0.920 ± 0.113 and 1.003 ± 0.125 μmol/g ww/2 h (mean $\pm$ S.E.M.) respectively. The difference between the two was not significant ($p > 0.1$) when tested by the Welch procedure which approximates the Student's t test for cases of unequal variances (11).

Table II demonstrates the effect of 0.1 and 1.0 μg/ml of PGE_1 on basal lipolysis. In none of the two types of adipose tissue did any significant reduction of glycerol release occur. The addition of noradrenaline or isopropylnoradrenaline stimulated lipolysis in the control tissue while, in the tissue from the hypothyroid subjects, only isopropylnoradrenaline was lipolytic (Fig. 1).

The effect of PGE_1 on lipolysis induced by noradrenaline and isopropylnoradrenaline is shown in Fig. 2. PGE_1 (0.1 or 1.0 μg/ml) inhibited lipolysis stimulated by the lower concentration of nor

Table II. *Effect of PGE$_1$ on basal lipolysis in subcutaneous adipose tissue from controls and hypothyroid subjects*

	Glycerol release[a]					
	Control (= 5)			Hypothyroid (= 5)		
Additions		Mean diff.	p		Mean diff.	p
Basal	0.972±0.177	—		0.994±0.256	—	
PGE$_1$ 0.1 µg/ml	0.735±0.085	−0.238±0.128	N.S.	0.837±0.249	−0.157±0.248	N.S.
PGE$_1$ 1.0 µg/ml	0.751±0.069	−0.222±0.155	N.S.	0.655±0.170	−0.340±0.211	N.S.

µmol/g ww/2 h (mean ± S.E.). Basal medium – PGE containing medium.

adrenaline (0.3 10^{-6} M) in tissues from controls as well as hypothyroid subjects. At a higher noradrenaline concentration (3×10^{-6} M) PGE$_1$ did not significantly influence the lipolytic response in the control tissue. In contrast, the lipolytic effect of noradrenaline (3×10^{-6}) was enhanced in tissue from hypothyroid subjects, but only the high PGE concentration showed a statistically significant stimulation ($p < 0.02$). In the control as well as in the hypothyroid group, PGE significantly inhibited the lipolysis induced by isopropylnoradrenaline (0.3 or 3×10^{-6} M).

DISCUSSION

Recent publications from our laboratory (8) and other investigators (3) have demonstrated that human adipose tissue is equipped with both alpha and beta adrenergic receptors. Stimulation of beta adrenergic receptors accelerates lipolysis by increasing the formation of cyclic adenosine 3',5' monophosphate (cAMP) in the tissue. Alpha adrenergic stimulation results in inhibition of lipolysis, probably by diminishing the rate of formation of cAMP (4). Thus the lipolytic response to catecholamines having both alpha and beta agonistic properties (e.g. noradrenaline) is less pronounced than that of a nearly pure beta agonist like isopropylnoradrenaline (8).

The balance between the responsiveness of the two adrenergic receptors to noradrenaline shows some variation in normal human adipose tissue (8). In addition, it was recently found that this

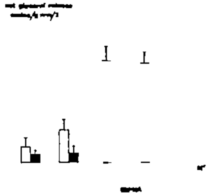


Fig. 1. Noradrenaline (NA) and isopropylnoradrenaline (ISPNA) (0.3 and 3.0 10^{-6}M)-induced lipolysis (mean ± S.E.M.) minus basal glycerol release in subcutaneous adipose tissue from five controls (open bars) and five hypothyroid subjects (black bars).

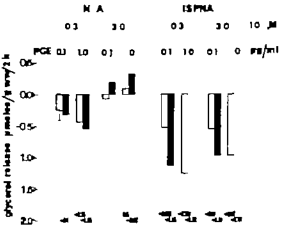


Fig. 2. Effect of PGE on catecholamine-induced lipolysis. The figure presents the difference in glycerol release (mean ± S.E.M.) induced by the adrenergic agonist alone and in the presence of PGE. The concentrations of noradrenaline (NA), isopropylnoradrenaline (ISPNA) and PGE$_1$ are indicated. Open bars = control subjects, black bars = hypothyroid subjects.

balance is markedly shifted towards a predominance of the alpha receptor in adipose tissue from hypothyroid subjects (14). Thus noradrenaline had no lipolytic effect unless the alpha receptor was blocked with phentolamine. No other major defect in the lipolytic machinery seemed to be present, since the responses to isopropylnoradrenaline, theophylline and dibutyryl-cAMP were within normal limits in this tissue.

Thus it seems that a diminished level of thyroid hormone has a modulatory action on the adrenergic receptor system of human adipose tissue.

The prostaglandins are another group of substances that has been discussed in connection with the modulation of adrenergic responses. These are naturally occurring hormones which, in many tissues, are believed to act by influencing the release and action of catecholamines (2). In man the infusion of small amounts of PGE_1 stimulates basal lipolysis while it reduces the lipolytic effect of noradrenaline (1). The degree of the antagonistic effect of PGE_1 on catecholamine-induced lipolysis varies when tested in vitro on human adipose tissue from healthy subjects and depends on whether a beta adrenergic agonist (isopropylnoradrenaline) or a combined alpha and beta stimulator (noradrenaline) is used. On the basis of these findings it has been suggested that PGE_1 acts by blocking the beta as well as the alpha adrenergic receptor responses (7)

Our results, showing that PGE_1 stimulated noradrenaline-induced lipolysis only in adipose tissue from hypothyroid subjects, while it blocked that of isopropylnoradrenaline in hypothyroid as well as in control tissue (Fig. 2), are in keeping with the above suggestion. With the knowledge that adipose tissue from hypothyroid subjects has an enhanced alpha receptor response, it can be deduced that the inhibition of this receptor by PGE_1 is so pronounced that noradrenaline becomes lipolytic in spite of a concomitant beta receptor inhibition by PGE_1

The above discussion implies that the inherent property of PGE_1 to inhibit both types of adrenergic receptor responses will express itself functionally in a varying manner depending on the initial balance between the receptors. The variability of PGE_1 effects described in the literature may in some tissues reflect variations in the state of the adrenergic receptors rather than true differences in the action of this hormone per se.

The mechanism for the adrenergic receptor inhibition by PGE_1 is not known. However PGE_1 demonstrates a number of effects, such as stimulation of glucose uptake (5), enhancement of Ca^{++} uptake and effect (12, 13) and regulation of water flux (9) Whether any of these or similar processes account for the effect of PGE_1 on the adrenergic system is a matter of speculation.

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macroglobulins, notably in glomerulonephritis, might be explained by metabolic differences rather than by renal factors.

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HEPATIC LIPOGENESIS IN TWO CASES WITH INSULIN-PRODUCING TUMOR OF THE PANCREAS

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Abstract. The incorporation rate of glycerol-1,3-^{14}C and fructose-U-^{14}C into glycerides and into the fatty acid moiety of glycerides has been determined in liver slices obtained from liver biopsies taken at operation of two patients with insulin-producing tumor of the pancreas. Both of these patients had high plasma concentration of insulin. The results were compared with the corresponding incorporation rate in liver slices obtained from fasting controls and controls pretreated with 400 g fructose on the day before operation (gallstone patients). In the insulinoma cases the incorporation rate and the distribution of label amongst liver lipids was in conformity with the fasting controls. A changed distribution of label with an increased incorporation of glycerol as well as fructose into the fatty acid moiety of glycerides was found in the patients pretreated with fructose. The results suggest that chronic hyperinsulinemia without increased concentration of plasma glucose does not promote hepatic lipogenesis in man.

Diet rich in low molecular carbohydrates produces an increase in the plasma triglyceride level in man (1, 7, 13) as well as in the experimental animal (6, 17). This rise is associated with an increased turnover of the plasma triglycerides including an accelerated influx of triglycerides from the liver (15, 19, 25).

Recently we reported a significantly increased incorporation of glycerol-1,3-^{14}C and fructose-U-^{14}C into triglycerides and into the fatty acid moiety of glycerides in liver slices obtained from patients operated on for uncomplicated gallstone disease when these patients were preoperatively (12 hours befor the operation) loaded with 400 g fructose intravenously (22).

The mechanism involved in the increased hepatic triglyceride synthesis and fatty acid synthesis in response to carbohydrate load is not known. However, it has been suggested that insulin promotes hepatic triglyceride synthesis (7, 20) and synthesis of glyceride fatty acids (9, 21). On the other hand, it has never been demonstrated that insulin stimulates hepatic lipogenesis in the absence of glucose supply, endogenous or exogenous (16), and according to Haft (11) high glucose concentration in the medium perfusing isolated liver effectively stimulates the lipogenesis even without insulin.

Patients with insulin-producing tumors and consequently with chronically high levels of plasma insulin offer a possibility to gain more insight into the effect of insulin per se on the hepatic lipogenesis. In the present work the incorporation rates of radioactive precursors into glycerides and glyceride fatty acids in liver slices from two patients with insulinoma and high plasma insulin levels are compared with the corresponding incorporation rates in fasting patients and in patients pretreated with fructose.

MATERIAL AND METHODS

Two male patients, aged 51 and 56 years, were admitted to the hospital on the suspicion of pancreatic insulinoma.

The first patient had suffered from symptoms of hyperinsulinism for 15 years—attacks of weakness, tremor of the hands and legs and profuse perspiration. The symptoms were rapidly cured by carbohydrate ingestion. During the last months the attacks had been more frequent. The following laboratory data were obtained in fasting state: plasma cholesterol, 270 mg/100 ml; plasma triglycerides, 1.72 mmol/l; plasma free fatty acids 1.2–1.9 mmol/l, blood glucose, 30–43 mg/100 ml; plasma insulin 37–61 μU/ml (mean of 5 determinations, 49 μU/ml). The normal plasma insulin value for randomly selected men at 50 years of age is 10 ± 2 μU/ml in our laboratory.

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Table I. *Percental distribution of incorporated label from glycerol-1,3-¹⁴C and fructose-U-¹⁴C amongst human liver glycerides (mean ± S.E.M.)*

Liver slices, 300 ± 3 mg, were incubated for 4 hours at 37°C in Hagedorn tubes. The incubation medium consisted of 3 % bovine albumin in Krebs–Ringer phosphate buffer. Palmitic acid complexed to 20 % albumin was added to reach a fatty acid concentration of 1.0 μmol/ml. Glycerol 5 μmol/ml and glycerol 1,3-¹⁴C (about 10^6 dpm) or fructose 5.5 μmol/ml and fructose-U-¹⁴C (about 4 × 10^6 dpm) were added as substrate. Final volume 4 ml and pH 7.4. Before incubation the tubes were gassed with 5 % CO_2 in O_2.
TG – triglycerides, PL – phospholipids, FA – fatty acids

	Glycerol			Fructose		
	TG	PL	FA-moiety	TG	PL	FA-moiety
Controls	70.1 ± 1.3	27.7 ± 1.2	0.9 ± 0.1	76.2 ± 2.6	21.7 ± 1.9	4.7 ± 0.7
Fructose-loaded controls	74.8 ± 1.2	20.7 ± 1.3	2.9 ± 0.5	79.1 ± 1.8	17.8 ± 1.3	16.0 ± 23
Insuloma cases						
Case I	68.2	27.2	0.5	84.1	15.9	4.3
Case II	63.5	28.5	0.9	79.4	20.6	3.5

$p < 0.005$. $p < 0.0025$ $p < 0.0005$.

On provoked starvation for 18 h the blood glucose concentration decreased from 65 to 24 mg/100 ml. Thereby the patient became confused but, following carbohydrate ingestion, he rapidly improved.

At operation a tumor, sized 2 cm in diameter was found in the pancreatic head. The tumor was excised with free margins. On microscopy a typical islet tumor of the pancreas was diagnosed by the pathologists. The postoperative course was uneventful and the patient has since been free from symptoms (8 months).

The other patient had been suffering from attacks of convulsions and unconsciousness during the last 6 months. These symptoms rapidly disappeared following carbohydrate ingestion.

The investigations revealed a probable islet tumor of the pancreas. The following laboratory data were obtained at fasting state: plasma cholesterol, 140 mg/100 ml, plasma triglycerides, 0.65 mmol/l; plasma free fatty acids, 0.9–1.4 mmol/l; blood glucose, 40–60 mg/100 ml, plasma insulin, 90–102 μU/ml.

At operation a tumor in the corpus part of the pancreas was found and a partial pancreatectomy was performed. On microscopy a typical islet tumor was diagnosed by the pathologists. The postoperative course was uneventful. Since the operation (4 months) the patient has been free from symptoms of hyperinsulinism.

The control group consisted of 37 patients, 12 men aged 47 ± 4 years and 25 women aged 43 ± 3 years. Of these patients 27 were admitted to the hospital for operation of nonobstructive gallstone disease, 8 for operation of benign gastric disease, and 2 for operation of hiatal hernia. All the patients had normal liver function judged from standard serum liver tests, and all of them were normo-lipidemic according to the following definition: serum cholesterol < 300 mg/100 ml, triglycerides < 2.0 mmol/l, and free fatty acids in serum < 1.0 mmol/l. At the time of operation and during the immediate preoperative period none of the patients had any disease of known influence on the study or had any medication known to affect the liver.

Eight of these control patients received 400 g fructose as an infusion during the day before operation. In these patients the caloric intake from the diet was diminished merely by reduction of the amount of food in each meal. The fructose infusion was ended at 8 p.m. All the control patients were fasted over the night before the operation.

The two patients with the diagnosis insuloma were given 1 000 ml of 10 % invertose i.v. over the night before the operation.

The patients were premedicated one hour before anaesthesia by means of morphine and atropine or analogous drugs according to the routine practice. The anesthesia consisted of hexobarbital (Evipan®), oxygen, nitrous oxide and succinyl-choline. The liver biopsy and the preparation of the liver slices were performed as previously described (18). The incubation of the liver slices and the determination of the incorporation rate of glycerol-1,3-¹⁴C and fructose-U-¹⁴C into liver lipids and CO_2 were performed as described in detail in previous papers (18, 22). (For experimental conditions see legend to Table I.)

The albumin used was bovine serum albumin (Armour, fraction V Batch H G 1371) extracted according to the method of Goodman (10). Palmitic acid was obtained from Mann. Res. Lab. USA, glycerol from E. Merck, West Germany, glycerol-1,3-¹⁴C and fructose-U-¹⁴C from New England Nucl. Corp. West Germany. Silica gel H (E. Merck, West Germany) was used for thin layer chromatography. Plasma insulin was radio-immunochemically determined according to Hales & Randle (12).

The lipid phosphorus determinations were performed according to Bartlett (3) as modified by Svennerholm (23). Glyceride-glycerol was determined as described by Carlson and Wadström (4). Neutral lipids and phospholipids were separated according to Gloster and Fletcher (8). Fatty acids were quantitated according to the description by Dole (5), as modified by Trout et al. (24). Determination

of the incorporation of label into the fatty acid moiety of glycerides was performed as previously described (18). The counting of radioactivity was performed in Packard-Tri-Carb (3329) liquid scintillation spectrometer.

RESULTS

The distribution of the incorporated label amongst lipids is given in Table I. In the control patients given fructose preoperatively the relative incorporation of labelled glycerol into triglycerides was significantly increased and into phospholipids significantly decreased as compared with the fasting controls. In these fructose-treated patients the relative incorporation of label from glycerol as well as from fructose into the fatty acid moiety of glycerides was more than three times higher than the corresponding incorporation in the fasting controls.

In the two patients with insuloma and high levels of plasma insulin the relative incorporation of label into the fatty acid moiety of glycerides from both substrates was of the same order of magnitude as in the fasting controls. The relative incorporation of labelled glycerol into triglycerides and phospholipids in the insuloma cases was also the same as in the fasting controls. The relative incorporation of labelled fructose into glycerides in these cases was in conformity with the control groups.

The mean ratios between the incorporation of label into CO_2 and into glycerides were 1 1 with glycerol as substrate and 4 6 with fructose as substrate for the fasting controls. The corresponding ratios for the fructose-loaded controls were 1.3 and 3 9 *and for the insuloma cases 1.3 and 2.8.*

The incorporation rate of glycerol into phospholipids was significantly lower and the incorporation rate of fructose into triglycerides significantly higher in liver slices from the fructose-loaded controls than from the fasting controls (Table II). The incorporation rate of glycerol into liver glycerides in the cases with insuloma did not differ from that in the fasting controls, and the incorporation rate of fructose into glycerides in these patients was of the same order of magnitude as in the controls.

The concentration of liver lipids in the insuloma cases was within the ranges found in the control material (triglycerides, 5 7 ± 1 4 μmol/g liver phospholipids, 23 1 ± 0.8 μmol/g liver).

Table II *Incorporation rate of glycerol into triglycerides and phospholipids in human liver slices (mean ±S.E.M.)*

The experimental conditions as indicated in Table I

	Glycerol (μmol/g liver/h)		Fructose (μmol/g liver/h)	
	TG	PL	TG	PL
Controls	0.31 ± 0 02	0.13 ± 0 01	0.09 ± 0 01	0 02
Fructose-loaded controls	0 27 ± 0 02	0.08 ± 0.01	0.11 ± 0 01	0 02
Insuloma cases				
Case I	0.28	0.11	0.12	0.02
Case II	0 32	0.13	0.12	0 03

$p<0\ 05$. $p<0\ 0025$.

DISCUSSION

The influence of insulin on the hepatic lipogenesis is debated. The close interrelationships, on the one hand, between the carbohydrate metabolism and insulin and, on the other between carbohydrate and lipid metabolism make it difficult to evaluate the importance of a single abnormality in the genesis of carbohydrate-induced hypertriglyceridemia. Results from animal experiments and clinical data provide evidence that insulin might promote fatty acid synthesis and triglyceride synthesis in the liver (7 9 20 21).

It has been shown that treatment of diabetic rats with high doses of insulin increases the activity of liver enzymes involved in the metabolization of carbohydrate and in the synthesis of fatty acids above the level of fed normal rats (26). Such an overshoot, which also has been shown for the *incorporation of labelled glucose into hepatic fatty* acids (14), suggests that insulin stimulates the hepatic lipogenesis. However it has to be emphasized that these results were obtained in dia-betic state. The metabolic consequences of chronic hyperinsulinemia in a nondiabetic organism might be quite different from those observed on substitution of insulin deficiency

Our results in the cases with insulin-producing tumor of the pancreas are to some extent contradictory to the general opinion on the effects of insulin on the hepatic lipogenesis. Both of our patients with insuloma had a long history of hyper insulinism and, during the preoperative period, tremely high concentrations of

reactive insulin in plasma were found. None of these patients had hypertriglyceridemia or steatosis of the liver i.e. there were no indirect signs of increased hepatic lipogenesis. In incubated liver slices from these patients the distribution of incorporated label from radioactive glycerol and fructose amongst liver glycerides was the same as in the fasted controls. Thus the incorporation of label into glyceride fatty acids was not increased. On the other hand, in control patients pretreated with fructose, the distribution of label was changed. In these patients a threefold increase of the incorporation into the fatty acid moiety of glycerides was found. In a previous report on this finding we suggested that this shunting of fructose and glycerol into the fatty acid synthesis pathway might be due to an increased level of plasma insulin (22). Based on the present results in the insuloma cases this explanation seems less probable. A further argument against this explanation is that dietary fructose is at least as active as glucose on hepatic lipogenesis despite its weak action on insulin secretion, and according to Baker et al. (2) it remains effective also in the absence of insulin

Salans and Reaven (21) reported recently that insulin pretreatment of fed normal rats led to a preferential incorporation of labelled glucose into triglycerides instead of oxidation in liver slices, provided that the incubation medium contained a high concentration of glucose. The low ratio between the incorporation of fructose into CO_2 and into glycerides in liver slices from our insuloma cases as compared to the corresponding ratio in fasting controls agree well with this finding of Salans and Reaven (21). However this shunting was not associated with increased incorporation of label into the glycerol or fatty acid moieties of glycerides in our cases.

Our results seem to indicate that chronic hyper insulinemia without increased plasma concentration of glucose does not promote hepatic lipogenesis in man.

ACKNOWLEDGEMENT

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PHYSICAL WORKING CAPACITY AND AGE

An Investigation in Conjunction with the Vasa Ski Race ("Vasaloppet")

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Abstract. An investigation of the performance of nearly 7 700 participators in long distance skiing race ("Vasaloppet") shows very regular change with rising age. The performance rises up to 30–35 years of age and thereafter falls continuously—at rate of approximately 6–7%/10 years.

Increasing numbers of people all over the world have begun to realize the importance of regular physical activity. This is particularly important for the large and growing section of the population who, except at their own initiative, get no exercise but have a sedentary occupation at a desk or machine and travel to and from their work by car or bus. In Sweden in the last few years this has led, for example, to mass participation in athletic competitions, especially cross-country running and skiing.

It is then important to investigate how much a person can accomplish at different ages and to get people who take part in competitions of these kinds—as also in more ordinary forms of physical exercise—to realize that one competes not only with others but, in particular, against one's own age. A unique means of making a large-scale investigation of the relation between physical working capacity and age was offered when the times for the Vasa Ski Race began to be recorded and calculated with a computer. The Vasa Race, said to be the longest race in the world and that which attracts the greatest number of competitors, extends over a distance of 85 km in hilly terrain. Until recently the number of participators was limited and consisted almost exclusively of elite skiers. In the past few years the number of starters has risen very rapidly.

MATERIAL

In 1970 the initial number of entries was 9 400, of whom about 8 600 appeared at the starting-post. More than 900 fell out of the race, which in that year was unusually difficult owing to the varying weather conditions. Data of year of birth were lacking for small number of competitors (~75) with recorded times for the race. This loss consists mostly of elite skiers, whose entry-forms do not contain complete age data etc. As result the material for this study comprises 7 625 persons.

All competitors start at the same time. By tradition, only men have been allowed to participate hitherto. The competitors include representatives of the top class of skiers as well as persons of all ages ([illegible] years) and from all professions. They vary greatly in training. Many, probably most, have engaged in methodical training over longer or shorter period; some enter the race without special training.

The material has been divided into 5-year groups. Conventional statistical analysis has been used.

RESULTS

The age distribution of the competitors will be seen from Fig. 1. The results, i.e. the times for the race in relation to age, are shown in Table I and in Fig. 2. Two findings in particular are of interest. In the first place the lowest part of the curve, i.e. the best times, is occupied not by the youngest but by competitors aged 31–35 years. In the second place the skiing times rise from that point practically linearly with growing age.

DISCUSSION

The physical working capacity of adults falls with growing age. Several investigators (1, 3, 4) have shown that the maximum oxygen uptake falls and,

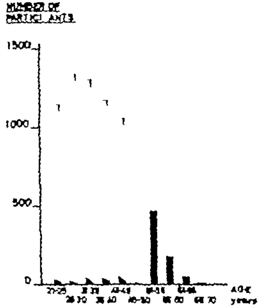


Fig. 1. Age distribution of the competitors (5-year groups).

in parallel with it, the maximum work that can be performed during a few minutes or longer period.

Skiing is a sport which engages large muscle groups and therefore places heavy requirements on the individual's oxygen uptake capacity. Provided that the skiing technique is reasonably developed, oxygen uptake is actually the factor which mainly determines performance. Oxygen uptake capacity attains its maximum at 18–20 years of age and thereafter falls at a uniform rate in an untrained

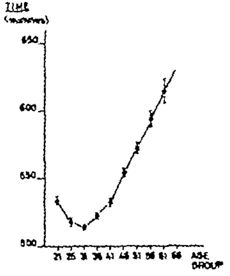


Fig. Skiing times in relation to age. Mean times of each 5-year group. (Statistical variables given in Table 1.)

Table I. *Results of the Vasa Ski Race in relation to age*

Age group (y.)	No. of competitors	Skiing times (min)		
		Mean	S.D.	S.E.M.
21–25	1 150	533	88	3
26–30	1 338	517	90	3
31–35	1 313	513	87	2
36–40	1 183	522	84	2
41–45	1 061	532	81	3
46–50	883	554	78	3
51–55	466	572	76	4
56–60	169	594	74	6
61–65	52	615	63	9
66–70	9	633	74	25

or little trained normal population (Fig. 3). A well trained individual, however, may maintain his maximum oxygen uptake at a high level up to 30 or even 35 years of age.

A significant factor is also the ability of the well trained individual to utilize his capacity. A close correlation has been found between maximum oxygen uptake, as determined in the laboratory and the work load spontaneously chosen by the individual. In building workers this spontaneous work load lies at around 40% of the maximum (2). A well trained individual can for long periods maintain a considerably higher load, corresponding to 70%, perhaps 80% of maximum oxygen uptake and thus partly compensate for an age-conditioned lowering of his maximum oxygen uptake.

The importance of physical training is revealed, for example, by investigations by Grimby and Saltin (8). They studied active cross-country runners who had been in training for several decades. Their oxygen uptake was about 30% higher than the normal values reported by I. Åstrand (1).

When training stops, those who have really trained thoroughly and over a long period will nevertheless retain a higher level of oxygen uptake than those who have never trained (10).

The results from the Vasa Race show that physical working capacity cannot be regulated solely by maximal oxygen uptake. The youngest competitors (20–30 years) had worse times than the group aged 31–35 years, although younger persons may generally be expected to have a higher maximal oxygen uptake. The difference in times between the groups is strongly significant ($p < 0.001$). The times thereafter deteriorate in accordance with a very regular pattern with rising age.

The results thus show that maximal skiing performance occurs at a later age than that for maximum oxygen uptake in a normal population. Fig. 3 shows a mean curve for oxygen uptake (3), together with the results for the race. For ease of comparison both have been converted to percentages (of maximum oxygen uptake and of shortest skiing time). Note that, in comparison with Fig. 2, the time curve is upside down—the best, that is the shortest time, i.e. maximum performance, is at the top of the curve. One sees that the reduction of performance and of oxygen uptake run parallel, but that the maximum oxygen uptake lies between 15 and 20 years against 30–35 years for maximum performance in skiing of this type.

The mean age of top athletes differs in different branches of athletics. Good sprinters are, on average, considerably younger than good Marathon runners (6)—technique and psychological factors play a greater role in the older person and may outweigh reduction of oxygen uptake. As already pointed out, furthermore, it is not certain that the maximum oxygen uptake of the competitors in the Vasa Race follows the normal curve. In many especially in the best trained, it may be expected to remain at a considerably higher level up to 30 years of age. The results in this large material, however seem to suggest that maximum performance in long-distance skiing occurs at a later age than that for maximum oxygen uptake—even if the difference is perhaps not fully as great as might appear from Fig. 3

The results obtain a greater interest through still another comparison. Fig. 3 shows (also in percentages) the maximum daily earnings of Swedish lumberjacks paid on the piece-rate principle. The figures are based on the earnings of more than 5 000 lumberjacks during the years 1947–1957 (9). It is found that the Vasa Race results change with age in exactly the same way as the daily earnings of lumberjacks, i.e. the results improve up to 30–35 years of age and thereafter fall continuously It may be added that the median time for the Vasa Race in 1970 was 8 / hours (510 min), i.e. the Vasa Race corresponds in time to a normal day's work, though of an altogether different and more persistent character

The Vasa Race competitors consist of persons from all social classes and with extremely varying degree of training, as also very varying physical activity in their daily work. The uniform variation

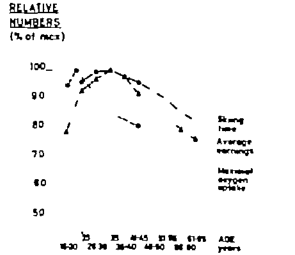


Fig 3. Comparison between maximal oxygen uptake (O O), skiing times in the Vasa Race (●——●) and average earnings of Swedish lumberjacks (Δ – Δ) in relation to age. 100% is the value for the highest oxygen uptake, the highest average earnings and the average skiing time for the best performing group—all other mean values are expressed as percentages of these highest (lowest) values. For further particulars see text.

in the results and the close correspondence with the results from the lumberjack investigation suggest that the figures are really representative of the lowering of physical working capacity with age.

The individual variation is very great. The fact that the physical working capacity of the average population falls with age does not imply that older individuals cannot occasionally retain a physical working capacity which is as good—or even better—than that of younger persons. Apart from congenital constitution, which is manifestly needed for really maximum performance—it is not everybody who can be trained to such levels of oxygen uptake as has been found, for example, in some of the Swedish skiing aces (up to 6 l/min). The individual capacity for maximum physical effort is affected, as noted, also by factors such as muscular strength, technique, and psychological factors. There is reason to suppose that the significance of these various factors may differ in different situations and at different ages, and that they may vary independently in relation to one another Our knowledge of these conditions today is very slight.

As already pointed out, in skiing the oxygen

uptake of the individual, as an expression of the central heart–lung function, should be an essential factor determining performance. But it has also been discussed whether the limiting factor might be of a peripheral and not of a central nature. Strandell (11) examined 140 healthy men aged 20–83 years and found that the maximum working capacity was best correlated to the lactate concentration in plasma. He interpreted this in the manner that the limiting factor in maximum work is of a peripheral nature. In support of this he found, too, that—in tests on a bicycle ergometer at a given load—both mean oxygen uptake and heart rate were the same in young and elderly healthy subjects.

A factor which may possibly be of significance for this peripheral limitation is, as recently demonstrated, that the content of triglycerides in skeletal musculature increases with age irrespective of the content of triglycerides in serum (7). A negative correlation has also been shown between "total work load" on a bicycle ergometer and the triglyceride content in musculature ($r = 0.53$, $p < 0.01$) (5). But it is difficult on the basis of the present facts to say anything definite about the significance of these findings.

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ADIPOSE TISSUE FAT CELL SIZE AND NUMBER IN RELATION TO METABOLISM IN ENDOGENOUS HYPERTRIGLYCERIDEMIA

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Abstract. Adipose tissue fat cell size and number have been determined in patients with endogenous hypertriglyceridemia. In addition to increased plasma triglycerides these patients were characterized, as previously well known, by decreased glucose tolerance, less frequently an increase in fasting plasma insulin and plasma insulin during glucose tolerance, as well as by moderate obesity. The obesity was due to increased fat cell size rather than fat cell number. In the hypertriglyceridemic patients statistical associations were found between fasting plasma insulin and fat cell size.

The association between endogenous hypertriglyceridemia and obesity has been demonstrated in a number of reports (1, 16, 21, 23) although there are exceptions (40). The nature of this association is, however, not clear (5). Patients preselected for obesity have increased plasma lipids, but in such populations with extreme obesity the elevation is not very marked (3, 15, 45). Weight-gain has been found to be an important factor accentuating this association (3, 21, 27). Furthermore, Albrink and Meigs (1) have pointed out that "acquired" rather than "natural" obesity is associated with increased plasma triglycerides. They also showed that adipose tissue in the hypertriglyceridemic obese patients was enlarged primarily on the trunk.

Recent findings of correlations between metabolic variables and adipose tissue cellularity seem to allow some clarification of this problem. Hirsch and Han (29) and Knittle and Hirsch (32) have demonstrated in studies in the rat that the number of fat cells in adipose tissue is apparently determined during an early period of life and remains stable. A variation in fat cell size rather than number has also been demonstrated in adult man (15, 30, 42). It is therefore likely that "acquired" obesity (1), defined as an increase of body fat in adult life, should mean an increase of body fat due to an increase in fat cell size. Such an increase of fat cell size has indeed also been found to be a frequent finding in moderate obesity (hypertrophic obesity) in which the obesity has been found to develop after childhood (15).

Plasma insulin is frequently elevated in endogenous hypertriglyceridemia (20, 22, 40). In hypertrophic obesity (15), in randomly selected middle-aged men (9, 10) and in men with myocardial infarction (4), as well as in dystrophic myotony (14), fat cell size has been found to correlate positively with fasting plasma insulin and glucose-stimulated plasma insulin. The previously reported association between endogenous hypertriglyceridemia and acquired obesity (1), the presumed adipose tissue cellularity of this condition, and the repeatedly found correlations between hypertriglyceridemia and hyperinsulinemia, and also between fat cell size and plasma insulin, made it probable that the obesity factor associated with hypertriglyceridemia may be an increase in fat cell size. It was therefore considered of interest to investigate this problem in order to clarify the association between obesity and hypertriglyceridemia. This is furthermore stressed by the fact that increased fat cells with associated hyperinsulinemia may occur without obvious increase in body fat (8). This would perhaps provide a link between endogenous hypertriglyceridemia and subjects who are not obese in conventional terms of body weight or body fat (40).

In the present work this question was subjected to an analysis in patient groups preselected

Table I. *Results of determinations in patients with endogenous hypertriglyceridemia and controls (means ± S E.M.)*

Groups		Age (y)	Body weight (kg)	Body fat (kg)	Fat cell weight (μg)	Fat cell number 10^{10}	Fasting insulin (μU/ml)	Sum of insulin (μU/ml)	Fasting glucose (mg/100 ml)
Men									
Controls	49	55	75±2	16±1	0.59±0.04	3.2±0.2	9±1	296±38	65±3
Patients	29	49±2	91±4	27±2	0.76±0.05	3.7±0.4	16±2	452±45[a]	86±6
p		–	<0.001	<0.001	<0.01	N.S.	<0.01	<0.05	<0.01
Women									
Controls	23	52	70±3	19±1	0.62±0.02	3.3±0.2	5±1	223±34	71±2
Patients	11	50±4	77±5	24±2	0.78±0.05	3.3±0.4	15±5	295±91[b]	91±9
p		–	N.S.	<0.05	<0.01	N.S.	<0.001	N.S.	<0.05

[a] Only 20. [b] Only 5

because of an increase in plasma triglycerides and pre β-lipoproteins identified by lipoprotein electrophoresis.

MATERIAL AND METHODS

Forty patients, 29 men and 11 women, aged 49±2 and 50±3 (means ±S.E.M.) years respectively were examined. They were referred to the clinic for investigations and treatment f hyperlipidemia (20 pats) found in follow-up investigations after myocardial infarction (7 pats.) or in population studies (3 pats.). Six patients were detected in investigations of obesity and four in investigation of dystrophic myotony They were selected by plasma triglycerides (17) and cholesterol (18) and paper electrophoresis of plasma lipoproteins (33). All the patients investigated had triglyceride values exceeding 150 mg/100 ml plasma without concomitant elevation of cholesterol and distinct pre-β-lipoprotein band on paper electrophoresis According to the classification suggested by Fredrickson et al. (23) they belonged to type IV

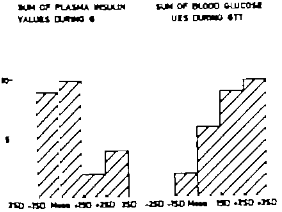


Fig 1 Distribution of sum of plasma insulin and blood glucose values during glucose tolerance test in patients with hypertriglyceridemia in relation to the means and S.D.s of control men and women.

All patients were subjected to investigation of the amount of body fat either by direct determinations of total body water and total exchangeable potassium (36, 37) with calculations of body fat according to Moore et al. (38) or in the 20 patients referred because of hyperlipidemia, by utilizing anthropometric data and regression equations valid for middle-aged men or women in the same geographical region as the patients (8 9). Fat cell size was determined in men in the abdominal subcutaneous fat layer below and to the left of the umbilicus and in women on the front of the thigh. In obese patients these two locations were also supplemented by determination in the gluteal region and the values were averaged. The representativity of these sites for the fat cell size of the whole body seems to be acceptable for the populations tested (8, 15). At least this fat cell size allows comparisons between groups, because fat cell size in different locations is strongly correlated within populations (6, 15). Fat cell size determinations were performed by percutaneous biopsy technique (28) and formalin fixation, freeze-cutting and microscopic measurements (43). Average fat cell weight was obtained as described by Goldrick (25). Total fat cell number was then estimated by dividing total body fat by fat cell weight.

Fasting heparin plasma insulin (26) and blood glucose (34) were determined concomitantly with plasma lipids. In most of the patients an oral glucose tolerance test (100 g glucose) was also performed, with blood glucose and insulin determinations at 30, 60, 90 and 120 min after the ingestion of glucose.

Randomly selected middle-aged men (9 44) and women (8) from Gothenburg were included for comparison. These control groups were examined in the same way as the patients with endogenous hypertriglyceridemia and have been analysed and described previously (4, 8, 9 10).

Statistical calculations were performed with Student *t*-test or with conventional linear regression analyses.

RESULTS

Table I shows the results of measurements in patients with hypertriglyceridemia in relation to

Sum of glucose (mg/100 ml)	Triglyceride (mg/100 ml)	Cholesterol (mg/100 ml)
455 ± 28	116 ± 6	259 ± 6
631 ± 35	270 ± 34	281 ± 9
<0.01	<0.001	<0.05
434 ± 12	80 ± 6	251 ± 10
214 ± 109*	214 ± 22	255 ± 26
<0.01	<0.001	N.S.

the controls. The men in the patient group had higher body weight and body fat than control men. The body fat increase was explained by an increase in fat cell size, but not in number. Insulin and glucose values were both higher in the hypertriglyceridemic group.

Also the hypertriglyceridemic women had higher body fat than controls, explained by an enlargement of fat cells but not by an increase in number of fat cells. Insulin values were elevated, although not significant for the sum of insulin values. When women and men were compared together the hypertriglyceridemic patients were characterized by increased insulin ($p < 0.05$) (Fig. 1). Blood glucose was also elevated in hypertriglyceridemic women.

Fig. 1 shows the distribution of insulin and glucose values of the hypertriglyceridemic patients during glucose tolerance test in relation to the means and standard deviations of the control materials. It is seen that an increased blood glucose value was a more frequent finding than an increased insulin concentration. However none of these elevations was apparently obligatory because some patients had values below the average of the controls.

Table II shows the correlations between different variables in men and women with endogenous hypertriglyceridemia. Fat cell size correlated positively and significantly with fasting insulin in women and in the whole material. There were no correlations between triglycerides and fat cell diameter. Sum of insulin showed a weak positive correlation with triglycerides in the whole material. No significant correlations were found between sum of insulin and fat cell diameter or between sum of glucose and triglycerides.

DISCUSSION

Patients preselected for hypertriglyceridemia were characterized as a group by decreased glucose tolerance and increased plasma insulin, and also by increased body f t in comparison with controls, as is well known from previous investigations (1 5 16, 21 23 24, 35). The increase of body fat was due to an enlargement of fat cells. The number of fat cells in adult life is probably stable (15 29 30, 32, 42). Variations of body fat in adult life are thus probably caused only by a variation in fat cell size (15 30 42) in turn caused by a variation in caloric balance. Increased fat cell size in hypertriglyceridemic patients thus indicates a positi e caloric balance, present or past, in obese patients. Albrink et al. (3) have previously pointed out the importance of weight increase for the elevation of plasma triglycerides.

Insulin during fasting and during glucose tolerance test was elevated in the patients with hypertriglyceridemia, but the frequency of patients with increased values was not as high as with de

Table II. *Correlations between different variables in men and women ith endogenous hypertriglyceridemia (n = 29 men, and 11 omen)*

	y	Men	Women	All	$p(t_0)$ Men	Women	All
Fasting insulin	Fat cell diameter	0.26	0.96	0.41	N.S.	<0.001	<0.05
Sum of insulin	Fat cell diameter	0.23[a]	0.06	0.16	N.S.	N.S.	N.S.
Triglycerides	Fat cell diameter	0.18	0.10	0.11	N.S.	N.S.	N.S.
Sum of insulin	Triglycerides	0.28[a]	0.18[b]	0.30	N.S.	N.S.	<0.10
Sum of glucose	Triglycerides	0.19[a]	0.34	0.16	N.S.	N.S.	N.S.

Only 20 Only 5

creased glucose tolerance. The insulin concentration is clearly higher than that found in patients with maturity onset diabetes mellitus (11) the latter having considerably lower triglycerides than the patients investigated in the present study This is in accordance with a previous suggestion (40) that, in association with decreased glucose tolerance insulin secretion is a prerequisite for the formation of constant hypertriglyceridemia.

The positive correlation between plasma insulin and fat cell size found in the present patients with hypertriglyceridemia has also previously been found in randomly selected men (9 10) in men after myocardial infarction (4) and in obese patients (15) It is noteworthy that this correlation was not found in randomly selected women (8) but was found in the present investigation in women with hypertriglyceridemia. The cause–effect relationship between these variables is not known. It has been suggested that insulin resistance in the enlarged fat cells might at least contribute to the general insulin resistance (41) Since the uptake of glucose in adipose tissue is very small (13) and since physical training can produce a decrease in plasma insulin in obesity without a change in fat cell size (12) it seems likely that the insulin resistance of enlarged fat cells is not the cause of hyperinsulinemia. It seems more likely that the hyperinsulinemia is caused by an insulin resistance in other tissues than adipose tissue, and that the increase of fat cell size is a secondary phenomenon to the hyperinsulinemia (7)

There is some evidence suggesting an increased peripheral insulin resistance in hypertriglyceridemia (19 31 39) This is partly counterbalanced by an increased insulin secretion, but apparently not fully corrected, because a decreased glucose tolerance still persists in spite of elevated plasma insulin. This condition thus seems to be associated with increased triglyceride accumulation both in adipose tissue and in plasma.

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MONOPHASIC ACTION POTENTIALS FROM RIGHT ATRIAL MUSCLE RECORDED DURING HEART CATHETERIZATION

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Abstract. Monophasic action potential (MAP) has been recorded in 32 patients using suction electrode technique. By repeated suctions total of 54 recordings were obtained which were acceptable for analysis. MAP is described in SR (34 cases), CSR (1 case), AF (4 cases), AFl (1 case) and A-arrest (2 cases). Comparison of MAP was possible between digitalized and undigitalized patients in SR. Acute digitalis effect was studied in 2 patients and acute atropine effect in another 2. Analyses of MAP was possible with respect to the duration of total MAP in all recordings and of phases 1–2 and 3 in 7 recordings. The duration of the MAP increases with decreasing heart rate during sinus rhythm. There is no statistical evidence of differences in MAP duration between patients with and without digitalis treatment. The variability in the duration of single MAP is associated with different relative repolarization rates of phase 3. Variations in duration of the MAP in individual patients are probably due to nonuniform vagal distribution. A short duration of the MAP in two patients with converted atrial fibrillation suggests the possibility of short refractory period which may reflect an arrhythmia tendency

In the ECG the P wave corresponds to the successive depolarization of the atrial muscle cells. Seldom is a T wave which reflects the atrial repolarization visible and most often it terminates during the ventricular depolarization. Thus electrocardiographic and vectorcardiographic studies are of very limited value for the interpretation of atrial repolarization.

Studies of the action potential (AP) and monophasic action potential (MAP) from atrial muscle have, however given valuable information about the local cellular events. Excellent reviews on this can be found in the extensive literature (7, 8, 24).

A few mainly methodological reports deal with MAP recording from the intact human atrium (12, 14, 18). Thus very little is known about the atrial repolarization in normal and abnormal conditions of the intact human heart.

In the present paper results of analyses of right atrial MAP recordings of the intact human heart are presented, using the MAP recording method described elsewhere (14). The results are compared with earlier knowledge of the electrical activity of atrial muscle. Some possibilities of the method in further studies of clinical pharmacological, physiological and pathophysiological problems are illustrated.

METHOD AND PROCEDURE

The present investigation was included as part of routine *diagnostic cardiac catheterization in 30 patients with* suspected heart disease and preferably normal sinus rhythm. Two further patients volunteered for the investigation. None of the 32 patients had received any premedication for the purpose of the investigation. During the heart catheterization, blood sample was aspirated from central venous catheter for analysis of serum electrolytes (Na, K, Ca, Cl, phosphate, bicarbonate, protein). An 11-lead ECG was recorded for control examination before the heart catheterization was performed. The MAP recording method utilized suction electrode catheter according to Olsson et al. (14). All recordings were done during supine rest. The MAP recording taken at each suction never exceeded two minutes. Several recordings were obtained by repeated suctions in each patient. Only those recordings which met the requirements described elsewhere (14) were accepted for analysis. This resulted in the variable number of recordings here presented for the individual patient.

Fig. 1 gives schematic illustration of the analyses of single MAP. Symbols in parentheses refer to the figure. The amplitude of the MAP was measured and expressed in mV (*I*). The duration of the MAP was measured at 90% repolarization and expressed in msec (*r*). Phase 0 of the MAP was defined as the rapid upstroke. Its duration as measured on the tangent from the time of 10% to the time of 90% of the MAP amplitude (*h*).

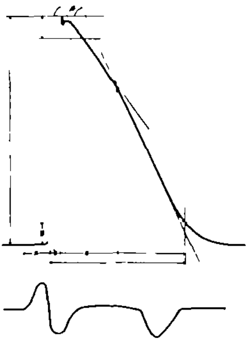


Fig 1 Analyses of single MAP For details see text. $h - g = 1/10\ f$

Obvious interferences from the close bipolar electrogram were corrected by hand. The duration of phase 3 was measured from the time when the tangents of phases 1 2 and 3 cross each other to the time of 90% repolarization of the MAP (*d*). The duration of phases 1–2 ( ) was determined by subtraction f the duration f phases 0 and 3 from the total duration of the MAP The relative repolarization rates (RRR) of phases 1–2 (tg α/f) and 3 (tg β/f) were calculated from the tangents of the different phases and expressed in %/sec of the total amplitude of the MAP The time interval from the beginning of the P wave in the right atrial electrogram to the onset of phase 0 of the MAP was measured and expressed in msec (*a*). The preceding electric cycle length as measured from the onset of phase 0 of the preceding MAP to the same event of the actual MAP and expressed in msec. All calculations were performed from five beats during which the cycle length was not allowed to deviate more than ±10%

MATERIAL

Altogether 32 patients were investigated. 4 of them had sinus rhythm, 4 atrial fibrillation, 1 coronary sinus rhythm, 1 atrial flutter and 2 atrial arrest. One of the 24 sinus rhythm patients (case 23) had developed atrial fibrillation during the investigation, and reverted spontaneously into sinus rhythm. In another of these 24 patients (case 27), sinus rhythm was restored by DC counter shock after atrial fibrillation hich had been discovered three months previously during a routine health investigation.

Eight of the remaining 22 patients had been on chronic digitalis medication for at least 6 months and one of *these patients also received quinidine therapy* due to ventricular premature beats (case 40). Two other patients (cases 14 and 33) received 1.2 mg Lanatosid C i.v one hour before the atrial MAP was recorded. The reason for this was study of ventricular MAP before and after acute digitalis. No atrial MAP was recorded in these cases before Lanatosid C administration.

The effect of 1.2 mg Lanatosid C i. was studied in 2 patients (cases 20 and 34) with sinus rhythm by re cording right atrial MAP before and 30 min after administration of the drug. In 2 patients without digitalis the acute effect of 0.5 mg atropine i.v was investigated (cases 21 and 25).

A total of 54 recordings of right atrial MAP were obtained from the 32 patients. Selected clinical data from all patients are given in Table I. The patients are numbered according to their consecutive order in the series of MAP recordings in the laboratory

RESULTS

Sinus rhythm

An example of a right atrial MAP during sinus rhythm is given in Fig. 2. The results of analyses of all MAP recordings during sinus rhythm and coronary sinus rhythm are presented in Table II. The patients are numbered according to the same principle as in Table I. No phase 1–2 is apparent in 39 of the 46 recordings showing that phase 3 determines the repolarization phase of the MAP Slight afterpotentials of the type depicted in Figs. 6 and 7 are seen in about 50% of the recordings. They are occasionally recorded in individual patients.

The time interval from the onset of the P wave of the right atrial electrogram to phase 0 of the MAP ranges between 0 and 60 msec in all recordings from the patients with sinus rhythm (Fig. 3) The corresponding values from



Fig. 2 Right atrial MAP and lectrogram (RAE) during sinus rhythm (case 38).

Table I. *Selected clinical data from patients investigated with right atrial MAP recording*

SR = sinus rhythm, NR = nodal rhythm, CSR = coronary sinus rhythm, AF = atrial fibrillation, Afl = atrial flutter VEB = ventricular ectopic beat, SVEB = supraventricular ectopic beat, RBBB = right bundle branch block, LAHB = left anterior hemiblock, A-arrest = atrial arrest, MI = mitral insufficiency MS = mitral stenosis, AI = aortic insufficiency AS = aortic stenosis, CMP = cardiomyopathy LAE = left atrial enlargement, LVH = left ventricular hypertrophy NM = not measured.

Case no.	Age (y.)	Sex	Diagnoses	Heart vol.	P_{PA} (mmHg) syst./diast. (mean)	P_{RA} (mmHg) mean (a wave)	ECG	Earlier arrhythmias	Electrolyte abnormalities
1	36	♂	MI	920/560	17/4 (9)	0 (3)	SR LAE LVH	0	0
2	55	♂	Lone AF	850/470	NM	NM	AF	AF	0
4	62	♀	MS	1180/690	41/17 (29)	4–5	AF RBBB	AF	K 2.0
5	29	♂	AS	680/350	24/4 (11)	2 (3)	SR	Rare SVEB	0
6	57	♂	Lone AF	900/520	NM	NM	AF	AF	0
8	61	♂	MS	650/400	RV 38/4	5 (9)	SR LAE	AF 3	0
9	48	♀	MI	770/420	24/9 (14)	2 (5)	SR	(arrhythmia feeling)	0
11	33	♂	Lone AF + dig. intox.	1020/550	15/5 (9)	2	NR RBBB LAHB A-arrest	AF A-arrest	0
12	63	♀	MS MI	790/530	49/29 (35)	5	AF	AF	0
14	56	♂	MI?	1000/570	27/12 (18)	6 (9)	SR LVH	0	0
16	54	♀	CMP	1050/610	32/15 (23)	2 (3)	SR	Sinus tachyc.	0
18	64	♂	AS	1160/580	24/10 (17)	2 (5)	SR SVEB VEB LVH	SVEB VEB	0
19	27	♂	AS	730/350	21/8 (15)	3 (4)	SR	0	0
20	50	♂	AS	750/400	22/9 (13)	1 (5)	SR	0	0
21	28	♂	Innoc. murm.	1000/420	18/5 (12)	1 (2)	SR	0	0
22	44	♂	Chron. VEB	620/330	17/8 (11)	2 (3)	SR VEB	VEB (tachy feeling)	0
23	55	♀	AS	1000/600	29/12 (18)	4 (9)	AF→SR LVH	Sinus tachyc.	0
24	41	♂	Constr. peric.	Not measurable	38/13 (21)	10 (13)	CSR	VEB	0
25	41	♂	Constr. peric.	1100/500	19/12 (15)	11 (14)	SR nonspec. ST T changes	0	Ca, P NM
27	59	♂	Lone AF	670/380	NM	NM	AF nonspec. ST T changes[a]	AF	Ca, P NM
28	50	♂	AI MI	1650/880	32/18 (24)	8 (15)	SR A–V block I LVH	VEB A–V block I	0
29	47	♂	CMP	1240/670	RV 78/13	13	NR A-arrest	AF NR VEB A-arrest	0
31	59	♂	AI AS	850/450	15/3 (9)	1 (2)	SR LVH	0	Ca, P NM
32	39	♀	Heart neurosis	590/310	18/9 (13)	4 (6)	SR	0	0
33	24	♀	Peric. calc.	560/340	23/14 (19)	6 (9)	SR VEB	VEB	0
34	39	♂	AS AI	720/400	26/11 (17)	3 (6)	SR	0	0
35	45	♂	AI MI	1140/540	22/8 (12)	1 (3)	SR LVH	(arrhythmia feeling)	0
38	54	♂	AI	1250/630	49/20 (30)	4 (7)	SR LVH	0	Ca, P NM
39	44	♂	AI	1450/780	32/12 (22)	1 (3)	SR LVH	VEB SVEB	0
40	53	♂	AS AI MS?	1280/670	43/17 (27)	3 (5)	SR LAE LVH	VEB	0
42	58	♀	MS	800/470	39/17 (26)	4 (7)	SR LAE LVH	AF	0
43	23	♂	Afl	450/260	RV 20/3	4	Afl	Afl	0

[a] After DC conversion SR LAE RBBB.

the patient with coronary sinus rhythm were 40 and 50 msec.

The amplitude of the MAP in all recordings during regular rhythm ranges between 3.1 and 17.7 mV (mean 8.2 mV, S.D. ±[illegible].9 mV). When repeated recordings have been performed, the variation coefficient of the amplitudes in a single patient varies between 3.6% and 37.4% (median value 11.0%).

The duration of the right atrial MAP ranges between 151 and 421 msec in regular rhythm. When repeated recordings have been made in a single patient under the same medication and heart rates differing less than ±10% of their

Table II. *Results of analyses of right atrial MAP during sinus rhythm and coronary sinus rhythm*

Rec. no.	Rhythm	Drug	P-ph0 (msec)	Cycle length (msec)	Ampl. MAP (mV)	MAP duration (msec) Total (ph0 + ph1 – 2 + ph3)	RRR ph2 (%/sec)	RRR ph3 (%/sec)
1B	SR		10	748	10.8	220 (5+)		448
5A	SR		10	573	17 7	220 (5+)		598
5B	SR		0	648	10.9	216 (5+)		645
8A	SR	Dig	19	816	6 9	268 (8+)		440
8B	SR	Dig	18	855	11 4	261 (9+)		461
9A	SR	Dig	20	683	*5.0*	*285 (12+)*		433
14E	SR	Dig	18	1 180	9 1	277 (5+)		433
16A	SR	Dig	0	772	6.1	246 (10+)		496
16C	SR	Dig	15	692	5 1	247 (5+)		378
18A	SR	Dig	38	815	5 8	327 (5+)		313
18D	SR	Dig	25	795	6.1	327 (10+)		331
19B	SR		35	745	7.2	296 (5+)		388
19C	SR		45	747	5 6	307 (5+)		369
20A	SR		40	755	6.3	270 (10+)		377
20F	SR		35	725	5.3	225 (10+)		450
20H	SR	Dig	30	826	7.8	300 (5+)		390
21A	SR		18	961	11.0	320 (15 + 155 + 150)	200	407
21C	SR		45	920	7 7	253 (10+)		439
21D	SR	Atr	28	691	13.4	264 (7 + 141 + 116)	207	435
21F	SR	Atr	38	683	12.6	250 (10 + 155 + 85)	286	577
22A	SR		18	625	7 4	259 (15+)		441
22B	SR		12	615	8.9	244 (20+)		655
23A	SR	Dig	0	700	4.6	151 (10+)		853
24A	CSR	Dig	40	834	7.6	297 (5+)		391
24D	CSR	Dig	50	889	10.3	313 (8+)		352
25A	SR		10	858	8.2	250 (15+)		426
25D	SR		24	831	5.8	316 (15+)		390
25J	SR	Atr	22	616	12.1	228 (22+)		552
27C	SR		35	1 164	6 5	183 (10 + 81 + 92)	171	736
28C	SR		33	768	5.9	267 (10 + 170 + 87)	311	553
31B	SR	Dig	39	1 086	7 4	298 (8+)		352
32A	SR		25	664	6.9	237 (10+)		474
33B	SR	Dig	15	852	9.9	251 (5 + 166 + 80)	394	601
34A	SR		28	914	11 1	256 (10+)		497
34C	SR	Dig	15	1 057	10 2	261 (10+)		463
34D	SR	Dig	20	968	10 1	290 (5+)		414
35C	SR		40	939	8 5	322 (10 + 159 + 153)	102	338
38A	SR		12	709	10 3	296 (10+)		414
38F	SR		18	676	7.5	276 (15+)		485
39A	SR	Dig	30	746	6.8	273 (10+)		381
40A	SR	Dig Quin	15	892	8 7	421 (7+)		254
40B	SR	Dig Quin	14	905	11 6	400 (4+)		213
42A	SR	Dig	40	666	3 3	285 (13+)		379
42B	SR	Dig	15	662	3 1	260 (10+)		453
42C	SR	Dig	60	686	5.6	254 (12+)		415

mean value, the variation coefficient of the duration of the right atrial MAP ranges between 0% and 16.5% (median value 3 8%).

Fig. 4 depicts the relationship between the duration of the right atrial MAP and the cycle length in all patients in sinus rhythm and free from atrial fibrillation during the investigation. When more than one MAP recording was done in the same patient during heart rates which differed less than ±10% from their mean values, the value in the figure is the mean of the different determinations. Cases 1 5 19 20, 21 22, 25 28 32, 34 35 and 38 who have been without digitalis treatment and free from atrial fibrillation during the investigation will be referred to as digitalis-free group" The patient group which is treated with digitalis but not quinidine and free from attacks of atrial fi-

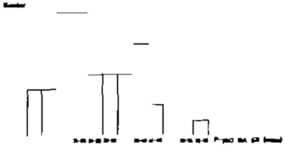


Fig. 3. Schematic presentation of time intervals from onset of P wave of right atrial electrogram to phase 0 of the MAP in all recordings during sinus rhythm.

brillation in connection with the investigation will be referred to as the digitalis-treated group (cases 8, 9 14 16, 18, 31 33 39 and 42). In patients in whom MAP was recorded before and after digitalis infusion or atropine injection only the values before administration of the drug are depicted in the figure

The equation for a mean square fitted regression line in the digitalis-free group is $Y = 0.16X + 138.5$ ($r = 0.60$, $S_{yx} = 27.1$ $\bar{y} = 264.8$) and in the digitalis-treated group $Y = 0.03X + 251.8$ ($r = 0.21$ $S_{yx} = 25.7$ $\bar{y} = 276.6$). In the two cases who had atrial fibrillation earlier during the investigation, the duration of the right atrial MAP is 151 msec (case 23) and 183 msec (case 27) respectively

The duration of phase 0 of the MAP ranges between 4 and 22 msec. In the 7 recordings where it was possible to distinguish between phases 1–2 and 3 the duration of phase 3 ranges between 80 and 153 msec and the duration of phase . between 81 and 170 msec.

Fig. 5 depicts the relationship between the RRR of phase 3 and the duration of the same MAP A strong correlation exists both in the digitalis-free and digitalis-treated group. The equation for the linear regression in the digitalis-free group is $Y = -1.74X + 923.5$ ($= 0.69$ $S_{yx} = 61.1$) and in the group treated with digitalis $Y = -_.38X + 1084.7$ ($r = 0.74$ $S_{yx} = 56.3$)

When the RRR of phase 3 is related to the preceding cycle length, the equation for the linear regression in the digitalis-free group is $Y = -0.41X + 777.2$ ($r = 0.59$ $S_{yx} = 68.4$) and in the digitalis-treated group $Y = -0.03X + 447.0$ ($r = 0.06$, $S_{yx} = 84.2$).

Acute digitalization results in prolonged cycle length as well as prolongation of the duration of the MAP This is illustrated in Fig. 6. Acute infusion of atropine is followed by increase of heart rate and mainly a shortening of the duration of the MAP This is illustrated in Fig. 7

No relationship has been found between the amplitude, duration or RRR of phase 3 of the MAP and the mean right atrial pressure or the a wave.

Coronary sinus rhythm

The values from the analyses of the right atrial MAP recorded in the patient with CSR (case with both digitalis and quinidine (case 40) is not included in the regression analysis.

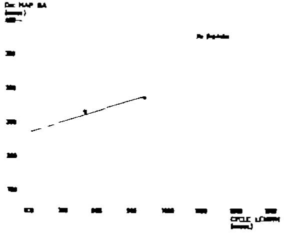


Fig. 4 Duration of right atrial MAP during sinus rhythm in relation to preceding cycle length. The patient treated

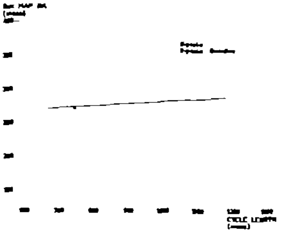


Table II. *Results of analyses of right atrial MAP during sinus rhythm and coronary sinus rhythm*

Rec. no.	Rhythm	Drug	P-ph0 (msec)	Cycle length (msec)	Ampl. MAP (mV)	MAP duration (msec) Total (ph0 + ph1 – 2 + ph3)	RRR ph2 (%/sec)	RRR ph3 (%/sec)
1B	SR		10	748	10.8	220 (5+)		448
5A	SR		10	573	17 7	220 (5+)		598
5B	*SR*		*0*	*648*	*10.9*	*216 (5+)*		*645*
8A	SR	Dig	19	816	6.9	268 (8+)		440
8B	SR	Dig	18	855	11 4	261 (9+)		461
9A	SR	Dig	20	683	5.0	285 (12+)		433
14B	SR	Dig	18	1 180	9 1	277 (5+)		433
16A	SR	Dig	0	772	6.1	246 (10+)		496
16C	SR	Dig	15	692	5.1	247 (5+)		378
18A	SR	Dig	38	815	5.8	327 (5+)		313
18D	SR	Dig	25	795	6.1	327 (10+)		331
19B	SR		35	745	7.2	296 (5+)		388
19C	SR		45	747	5 6	307 (5+)		369
20A	SR		40	755	6.3	270 (10+)		377
20F	*SR*		*35*	*725*	*5.3*	*225 (10+)*		*450*
20H	SR	Dig	30	826	7.8	300 (5+)		390
21A	SR		18	961	11.0	320 (15+155+150)	200	407
21C	SR		45	920	7 7	253 (10+)		439
21D	SR	Atr	28	691	13 4	264 (7+141+116)	207	435
21F	SR	Atr	38	683	12.6	250 (10+155+85)	286	577
22A	SR		18	625	7 4	259 (15+)		441
22B	SR		12	615	8.9	244 (20+)		655
23A	SR	Dig	0	700	4.6	151 (10+)		833
24A	CSR	Dig	40	834	7.6	297 (5+)		391
24D	CSR	Dig	50	889	10 3	313 (8+)		352
25A	SR		10	858	8.2	250 (15+)		426
25D	SR		24	831	5.8	316 (15+)		390
25J	SR	Atr	22	616	12.1	228 (22+)		552
27C	*SR*		*35*	*1 164*	*6.5*	*183 (10+81+92)*	*171*	*736*
28C	SR		35	768	5.9	267 (10+170+87)	311	553
31B	SR	Dig	39	1 086	7 4	298 (8+)		352
32A	SR		25	664	6.9	237 (10+)		474
33B	SR	Dig	15	852	9.9	251 (5+166+80)	394	601
34A	SR		28	914	11 1	256 (10+)		497
34C	SR	Dig	15	1 057	10 2	261 (10+)		463
34D	SR	Dig	20	968	10.1	290 (5+)		414
35C	SR		40	939	8 5	322 (10+159+153)	102	338
38A	SR		12	709	10 3	296 (10+)		414
38F	SR		18	676	7.5	276 (15+)		485
39A	SR	Dig	30	746	6.8	273 (10+)		381
40A	SR	Dig Quin	15	892	8.7	421 (7+)		254
40B	SR	Dig Quin	14	905	11 6	400 (4+)		213
42A	SR	Dig	40	666	3.3	285 (13+)		379
42B	SR	Dig	15	662	3.1	260 (10+)		453
42C	SR	Dig	60	686	5.6	254 (12+)		415

mean value, the variation coefficient of the duration of the right atrial MAP ranges between 0% and 16.5% (median value 3 8%)

Fig. 4 depicts the relationship between *the duration of the right* atrial MAP and the cycle length in all patients in sinus rhythm and free from atrial fibrillation during the investigation. When more than one MAP recording was done in the same patient during heart rates which differed less than ±10% from their mean values, the value in the figure is the mean of the different determinations. Cases 1 5 19 20, 21 22, 25 28, 32, 34 35 and 38 who have been without digitalis treatment and free from atrial fibrillation during the investigation will be referred to as digitalis-free group The patient group which is treated with digitalis but not quinidine and free from attacks of atrial fi-

be drawn concerning normal physiological limits of the parameters investigated. All patients have originally been suspected to suffer from heart disease and in all but two a definite diagnosis of heart disease was ultimately made.

The present method can never yield the exact information about the electrical events of a single cell that microelectrode studies can (9 14) Recordings of the electrical activity of single cells from the human heart using microelectrode technique have been performed during heart surgery (2, 25) but the technical difficulties have made the method unsuitable for more delicate studies. Many of the nonphysiological conditions that exist in studies of the electrical activity of single cells in preparations or during heart surgery have been partly overcome by the MAP recording method used.

The configuration of the AP from atrial fibers varies with among other factors species (8), cycle length (8), ionic composition of the surrounding fluid (7 8, 24) and in different fibers of the same auricle (3). The configuration of the MAP from the intact human heart can thus also be suspected to vary with several of these factors.

The general configuration of the MAP during regular rhythm is in agreement with the observations on human trial AP recorded in vitro by Trautwein et al. (21), Prasad (16) and Sleator et al. (20). Another type of human atrial AP with double spikes described by Prasad (16) and Sleator

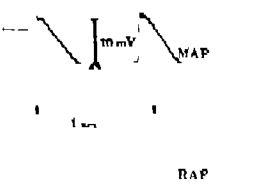


Fig. 8 Right atrial MAP and electrogram (RAE) during coronary sinus rhythm (case 24). Note the slow phase 4 depolarization.

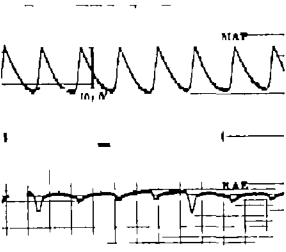


Fig. 9 Right atrial MAP and electrogram (RAE) during atrial flutter (case 43).

et al. (20) was not recognized in the MAP recordings.

Cellular electrolyte state could not be measured or assessed. Electrolyte determinations in serum (Na, K, Ca, Cl, phosphate, bicarbonate protein) were performed in the majority of the patients. The values were within normal limits except in case 4 with atrial fibrillation and hypopotassaemia. There is no cause to suspect any abnormalities of Ca and phosphate ions in the few patients where no determinations were performed. Thus it can be assumed that variation of MAP configuration which could possibly be attributed to different ionic composition of the blood was within physiological limits in patients with regular rhythm.

The present investigation has revealed differences in the duration of the right atrial MAP between different patients as well as in the same patient. As the duration of the MAP closely resembles the duration of the AP (9), which reflects the refractory period (8) a wide distribution of the refractory period of intact human atrial muscle is evident in the present material.

In the digitalis-free group the duration of the right atrial MAP is significantly correlated at the 5% level to cycle length and thus heart rate. A shortening of the right atrial MAP with decreasing heart cycle length in separate patients has earlier been pointed out by Shabetai et al. (18), but is not evident in the report by Korngren et al. (12). A similar correlation between cycle leng du

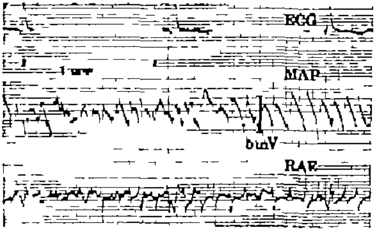


Fig 10 Precordial differential ECG, right atrial MAP and electrogram (RAE) during atrial fibrillation (case 2).

tion of right ventricular MAP exists and will be discussed more extensively elsewhere (15) However variations in the duration of the local electrical cellular events are more complex in the intact atria than in the ventricles. The vagal discharge influences the duration of the AP or MAP of atrial muscle cells (7 10, 11 13 17) but not of ventricular muscle cells of the intact dog heart (17) The effects of sympathomimetic amines on the atrial AP are considerably less pronounced than those of ACh (8) In vitro studies, where the nervous influence is absent, have revealed that a decreasing cycle length is associated with a shortening of the action potentials of mammalian atrial fibers (8, p 50) Also during a constant stimulation frequency the addition of ACh is followed by a shortening of the AP of mammalian atrial muscle (8, p. 54) Thus the increased vagal discharge that is associated with decreasing heart rate counteracts the effect of lowered heart rate on the duration of the AP of the intact atrium

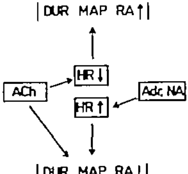


Fig 11 Some factors influencing the duration of the right atrial MAP of the intact human heart. For details see text.

(Fig. 11). The present investigation speaks in favour of the fact that the direct effect of cycle length alterations on the MAP duration is more pronounced than the effect of the different vagal influences in regular heart rates.

In several patients, the right atrial MAP has been recorded several times during almost identical heart rate. The recording places have not been identical, as indicated by the time from the onset of the P wave to phase 0 of the MAP The variation coefficients of the durations in these recordings are clearly in most cases higher than would be expected from the error of the method. Different durations of the right atrial epicardial MAP have been shown to represent different vagal influence in dogs (13) A nonuniform vagal distribution on the atrial muscle has also been demonstrated with pacing technique (1)

In the recordings obtained from right atrium close to the sinus node as indicated by short P phase-0-interval, the MAP duration is shorter suggesting more pronounced vagal influence more proximal to the sinus node. This is true for the majority of the multiple MAP recordings during similar heart rates and medication but the observation is not statistically significant according to tests of paired differences.

The duration of the MAP in the digitalis-treated group is statistically not different from the duration in the digitalis-free group. The vagus-stimulating activity of digitalis might be expected to shorten the duration of the MAP but the decreased heart rate will counteract this shortening. In addition to these effects, digitalis also has a direct influence upon the AP of a single muscle cell. The effect of digitalis on the duration of the

AP of isolated human atrial fibers has not been clarified and the effect on the duration of AP from other species are conflicting (8, p. 71 19). The sum of all these effects which are present in vivo cannot be predicted.

In the two patients where Lanatosid C is in fused and MAP recorded before and 30 min af ter infusion, the duration of the MAP is prolonged (Fig. 6). Whether this finding has general validity remains to be demonstrated.

Atropine injection can be expected to increase the duration of the MAP by the effect of inhibiting ACh action on the muscle cells (17). This is counteracted by the effect of the increased heart rate which follows atropine injection. In the two patients from whom MAP was recorded before and after atropine injection, there is mainly a decrease of the duration of the MAP (Fig. 7). A more extensive study is needed to analyse the effect of atropine on the MAP of the intact human heart.

One patient (case 40) was treated with quinidine as well as digitalis. The duration of the right atrial MAP was in this case considerably longer than in all other recordings. This is in agreement with the findings of Vaughan-Williams (22) who describes a prolongation of the terminal portion of the rat atrial action potential under the influence of quinidine, though the shape of the MAP in case 40 lacks prolongation of only the terminal part of phase 3

In two patients the MAP was recorded shortly after conversion of atrial fibrillation to sinus rhythm. In both these patients, the duration of the MAP was considerably shorter than in the other recordings. This suggests that the refractory period is also shorter in these cases as the dura tion of an AP is correlated to the refractory period (8) and a MAP and an AP have the same duration (9). A short refractory period favours the occurrence of an arrhythmia (23) and the atrial fibrillation episodes in these two patients may thus be explained by the short refractory period. It is possible that short duration of MAP is of value in predicting a tendency to atrial ar rhythmias (15).

The absence of a distinct phase 1–2 of the MAP in most recordings is in accordance with the observations on small pieces of human atrial tissue obtained during heart surgery (21). When no distinct phase 2 is present, the variation of the duration of the AP is to a high extent due to variability in repolarization rate of phase 3 This is nicely illustrated by Hoffman and Cranefield (8 Figs. 3–2 and 3–5). The strong correlation between the RRR of phase 3 and the duration of the MAP in the present study indicates that this effect on the repolarization rate is also true for the intact human heart.

The onset of phase 0 of the MAP represents the excitation of the muscle group under the elec trode. Thus the propagation of excitation of the heart can be explored by recording the MAP from different places and relating it to P-phase 0-interval. In the present study no exact localiza tion of the catheter tip was possible. Thus no atrial endocardial excitation mapping could be performed. The epicardial atrial excitation of two human hearts has been elucidated by Durrer et al. (5). They found that the excitation spreads more or less concentrically from the sinus node. As the excitation spreads along the atrial walls, the time difference between the excitation of the endocardial and epicardial surface of any specific part of the atrium must be very small. In all patients with sinus rhythm in the present study the P-phase-0-intervals lie within the excitation time intervals reported by Durrer et al., even when correction is made for the increased conduction velocities in their experiments.

The amplitude of a MAP is influenced by many factors (4). One of the factors which is of great importance is the thickness of tissue interposed between the electrode and the muscle cell group from which the MAP is recorded. In the right atrium, the subendocardial connective tissue layer is thicker than in the ventricle (6, p. 116). With the present technique, the amplitude of the right ventricular MAP is 10–50 mV (15). The lower amplitudes recorded from the right atrial endocardial surface can at least partly be explained by the different thickness of the subendocardial connective tissue layer An AP is in itself lower in atrial than in ventricular fibers (21). This probably contributes to the differences between the atrial and ventricular MAPs also.

In some patients the right atrial or pulmonary artery pressure has reached rather high levels. This can lead to right atrial endocardial thick ening (6, p. 417), which will cause a decreased MAP amplitude (4). However no distinct fall in the MAP amplitudes has been observed in

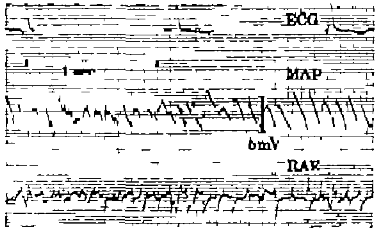


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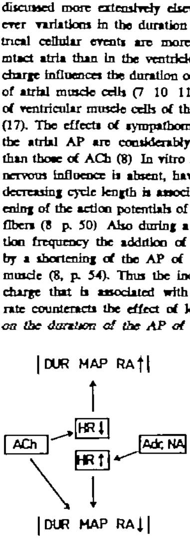


Fig 11 Some factors influencing the duration of the right atrial MAP of the intact human heart. For details see text.

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MONOPHASIC ACTION POTENTIAL AND SINUS RHYTHM STABILITY AFTER CONVERSION OF ATRIAL FIBRILLATION

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Abstract. Right atrial MAP has been recorded after conversion of atrial fibrillation in 11 patients, 10 of them after DC shock and 1 after spontaneously reverting attack of atrial fibrillation. The duration of the right atrial MAP has been of prognostic value for the 3-month maintenance of sinus rhythm. Three patients who maintained sinus rhythm more than 3 months had MAP duration in the same range as is found in patients free from atrial fibrillation. Six patients who relapsed into atrial fibrillation within 3 months had MAP duration lower than the arrhythmia-free population. In 2 patients MAP could *not be recorded during sinus rhythm. The short* duration of the right atrial MAP seems to reflect the arrhythmia tendency and can probably be explained by low atrial muscle potassium content.

Atrial fibrillation as an ECG diagnosis has been known in man since 1909 (21). Many types of heart diseases can lead to atrial fibrillation and in addition there are some cases in whom organic background to the condition appears to be lacking.

The risks of complications and functional consequences can be abolished by converting atrial fibrillation into sinus rhythm. Nowadays this is most often done by DC conversion. The ease with which this can be done has allowed more patients to be admitted for treatment. The difficulties are no longer related to the acute conversion but in the maintenance of sinus rhythm after successful conversion. Prediction of the long-term results of conversion to sinus rhythm is thus important and has been attempted with varying degrees of success in various ways (4, 13, 14, 24).

In previous work (17), it was observed that two patients with recent atrial fibrillation showed an unusually short monophasic action potential (MAP), recorded from the right atrial endocardial surface. The purpose of the present study is to evaluate whether or not the right atrial MAP can be of prognostic value in the determination of long-term results after DC conversion of atrial fibrillation.

MATERIAL AND METHODS

Eleven patients were investigated with right atrial MAP recording in connection with conversion of atrial fibrillation. Selected clinical data from all patients appear in Table I. The patients are numbered according to their consecutive order in the series of MAP recordings in the laboratory. Cases 23 and 27 are identical with those presented in previous paper (17). In case 23 the recording was performed 15 min after spontaneously reverting attack of atrial fibrillation, lasting 25 min, during routine heart catheterization. All other cases were admitted for therapeutic DC conversion of atrial fibrillation after at least three weeks treatment with dicoumarol and were all informed about the study. In these patients, quinidine tolerance tests had been performed some days prior to the DC conversion attempt according to the method described elsewhere (13). 50 mg Pethidine® was given as premedication 30 min prior to the DC conversion which was performed during light Brietal® anaesthesia. At the time of the conversion attempt, no patient was premedicated with quinidine, and digitalis treatment was stopped the day before the investigation. The DC shock was released between two electrodes, firmly pressed against the thorax, one at the apex area and the other at the base of the heart. The energy amounts required for restoring sinus rhythm varied between 100 and 400 joules.

The right atrial MAP was recorded from one place continuously up to two minutes with the exception for 10 to 15 sec during which the DC shocks were released and the input of the amplifiers used for MAP and electrogram recording disconnected. The MAP recording technique was the one described elsewhere (16).

The general pattern of the right atrial MAP recording

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Table I *Selected clinical data*

Case no.	Age (y)	Sex	Diagnosis	AF duration	Heart volume	P_{RA}	Medication	Electrolytes
23	55	♀	AS	25 min	1 000/600	4	Digitalis Lasix + KCl	Normal
27	59	♂	Lone AF	> 2 mo	670/380	NM	Dicoumarol	Normal (Ca, P NM)
44	40	♂	MI MS AS	3 weeks (earlier DC conv 4 times)	1 400/700	3	Digitalis Dicoumarol	Normal (Ca, P NM)
45	65	♂	Ess. hyp.	> 1 y (earlier DC conv once)	970/530	NM	Digitalis Hygroton + KCl Dicoumarol	Normal (Ca, P NM)
47	48	♂	MS MI	4 mo (tachycardia attacks for 5 y)	910/480	3	Digitalis Dicoumarol	Normal
48	66	♀	Ess. hyp. Ang. pect.	6 mo.	640/390	1	Digitalis Salures-K Dicoumarol	Normal
49	47	♂	Paroxysmal AF and Afl	8 mo.	820/370	1	Digitalis Dicoumarol	Normal
50	59	♂	Lone AF	> 3 mo. (earlier DC conv once)	860/400	3	Digitalis Dicoumarol	Normal
51	63	♀	Cardiosel.	1 mo (earlier DC conv twice)	950/560	3	Digitalis Dicoumarol	Normal
52	55	♂	Ang. pect. Chron. bronch.	> 3 mo	770/400	2	Digitalis Dicoumarol	Normal
64	49	♂	Paroxysmal AF (since 15 y)	3 mo.	900/460	4	Digitalis Dicoumarol	Normal

during atrial fibrillation immediately prior to the DC conversion attempt was compared with the appearance of the *f* waves in the ECG by visual inspection.

During sinus rhythm the amplitude of the right atrial MAP was measured and expressed in mV. The cycle length was measured from phase 0 of the preceding MAP to the same event of the MAP in question. The duration of the right atrial MAP was measured at 90% repolarization and expressed in msec. All calculations during sinus rhythm were performed from 5 beats during which the cycle length was not allowed to deviate more than ±10% of the mean value. The methods for analysing the MAP have been more extensively described elsewhere (17). In two patients right atrial MAP was recorded from three places after restored sinus rhythm, in all other patients from one place.

Serum electrolytes (Na, K, Ca, Cl, phosphate, bicarbonate, protein) were determined from blood sample taken during the investigation. A right atrial pressure was recorded in some patients before the conversion attempt using Statham Pb23Db transducer Beckman Dynograph R amplifier and an Ultralette 1610 recorder.

Patients 27, 48 and 49 revealed gastrointestinal intolerance symptoms to quinidine and were treated with digitalis only after DC conversion. Patient 47 who had gastrointestinal intolerance symptoms for quinidine and was treated with Inderal® 10 mg three times daily and Egazil Durules® 0.6 mg twice daily. Patient 44 was treated with digitalis plus quinidine after the conversion to sinus rhythm. The other patients failed to maintain sinus rhythm during the hospital stay. The patients were controlled as out patients during three months and the time of relapse into atrial fibrillation was noted.

RESULTS

Two patients (cases 45 and 51) failed to convert to sinus rhythm for even the few seconds necessary for the analyses of five heart beats during regular sinus rhythm. Three patients (cases 44, 48 and 49) maintained sinus rhythm more than 3 months and the other patients had relapsed to atrial fibrillation before the end of 3 months after the conversion. For further details see Table II. One patient with recurring attacks of atrial fibrillation (case 23) died during corrective surgery of aortic valves two months after the investigation. However during this time she had relapsed several times into attacks of irregular heart rhythm.

Right atrial pressure ranged between 1 and 4 mmHg. The pressure tracings all had normal configuration except for the absence of the *a* wave. In cases 27 and 45 no atrial pressure was measured however the clinical examination did not reveal increased venous pressure.

In the recordings obtained during atrial fibrillation course *f* waves in the ECG and high amplitude and long duration of the MAP sometimes coincided. This was, however, not a general finding. Fig. 1 depicts a MAP recording during atrial fibrillation in case 52.

Table II. *Results from analysis of MAP recordings during sinus rhythm (SR) and follow-up*

Case no.	Ampl. MAP RA (mV)	Cycle length (msec)	Dur. MAP RA (msec)	SR maintenance	Drug therapy after restored SR
23	4.6	700	151	14 d.	Digitalis, Lasix + KCl
27	6.5	1 164	183	< 1 mo.	Digitalis
44	7.4	719	233	> 3 mo.	Digitalis, Quinidine Dur 0.6 g
45	—	—	—	0	(Digitalis, Hygroton + KCl dicoumarol)
47	5.9	857	150	7 d.	Digitalis, Inderal 10 mg × 3
	6.1	911	195		Egazil Dur 0.6 mg × 2
	4.6	844	207		
48	4.3	686	280	> 3 mo.	Digitalis, Salures-K
49	9.2	638	345	> 3 mo.	Digitalis
	7.7	629	297		
	10.8	630	230		
50	15.0	506	144	10 min	(Digitalis, dicoumarol)
51	—	—	—	0	(Digitalis, dicoumarol)
52	8.3	592	197	4 min	(Digitalis, dicoumarol)
64	5.0	705	163	3 min	(Digitalis, dicoumarol)

The amplitude of the right atrial MAP during sinus rhythm ranged between 4.6 and 15.0 mV.

The duration of the right atrial MAP during sinus rhythm varied between 144 and 345 msec. Fig. 2 depicts a MAP recording during sinus rhythm in case 49. In the two patients from whom more than one recording was obtained during sinus rhythm, the variation coefficient of the duration of the right atrial MAP was 19.9% (case 49) and 16.3% (case 47).

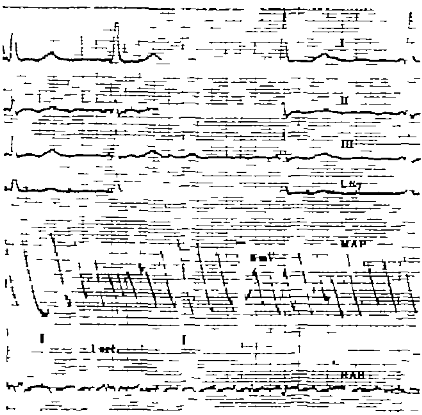


Fig. 1. Routine ECG leads, right atrial MAP and electrogram (RAE) during atrial fibrillation (case 52). The ECG leads are not uniformly amplified.

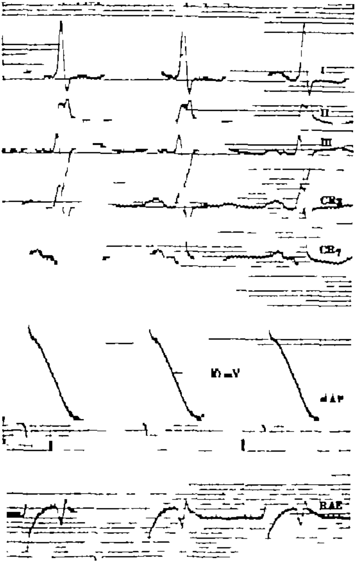


Fig 2. Routine ECG leads, right atrial MAP and electrogram (RAE) during sinus rhythm a few minutes after conversion of atrial fibrillation (case 49). The ECG leads are not uniformly amplified.

In the patients who maintained sinus rhythm more than three months, the duration of the right atrial MAP varied between 230 and 345 msec in the different recordings. In the patients who relapsed into atrial fibrillation before three months, the corresponding values ranged between 144 and 207 msec. Fig. 3 depicts the relationship between the duration of the right atrial MAP and the cycle length in all patients who were converted to sinus rhythm. The values given for patients 47 and 49 are the mean values of the three determinations in each patient as well as the individual values. The figure also presents the regression line and the 95% confidence limits from results of similar analyses of right atrial MAP during sinus rhythm in a population free from atrial fibrillation (17). This reference population is composed of all arrhythmia-free cases from the patient groups called digitalis-treated group and digitalis-free group but not the patient with both digitalis and quinidine.

DISCUSSION

Maintenance of sinus rhythm

The investigation has revealed that in this series of patients the right atrial MAP recording has been of prognostic value for the 3-month maintenance of sinus rhythm after conversion of atrial fibrillation.

In the three patients who have maintained sinus

rhythm more than 3 months the duration of the right atrial MAP in relation to the cycle length was above the lower 95% confidence limit of the corresponding values from a population free from atrial fibrillation. This reference patient group includes several patients who might develop atrial fibrillation later due to their heart disease; however, none has ever had atrial fibrillation prior to the investigation (17). The patients who have failed to maintain sinus rhythm after DC conversion have an unusually short duration of the right atrial MAP compared with those of the reference group.

Several different clinical details in the patient histories are of prognostic value for maintenance of sinus rhythm after DC conversion. In this small series of patients, there is no evident relationship between the heart volume and the maintenance of sinus rhythm after DC conversion. Neither is there any obvious relationship between duration of atrial fibrillation and persistence of sinus rhythm after conversion. These parameters have been of predictive value in larger patient groups, admitted for conversion of atrial fibrillation (4, 13, 14, 24), though without convincing applicability in individual patients.

A prolonged follow-up study would probably have resulted in a further diminished maintenance of sinus rhythm. However, several authors have reported that the relapses into atrial fibrillation mainly occur within 1–2 months after DC conversion (13, 14, 20, 24). Inadequate quinidine concentration might induce relapse to atrial fibrillation (4). The absence of quinidine treatment might have caused relapse into atrial fibrillation in patients who were not given the drug due to gastrointestinal intolerance symptoms.

Changes in the extracellular concentration of potassium or calcium can lead to cellular electrical phenomena which are predisposing to arrhythmias (8). Variations in the extracellular concentration of potassium influence the depolarization rate of phase 4 of the specialised conductive system while calcium acts by changing the level of the threshold potential on these cells. Serum electrolytes were normal at the time of the DC conversion attempts though in a few cases calcium and phosphate were not measured. However, there are no reasons for suspecting any abnormalities of these ions in the patients. *It is* thus not probable that the failure of establishing

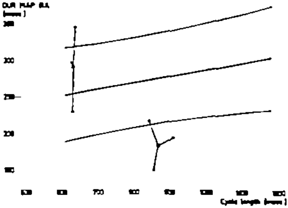


Fig. 3. Relationship between duration of right atrial MAP and cycle length in patients converted from atrial fibrillation to sinus rhythm, compared with mean square fitted regression line and 95% confidence limits from *results of similar analyses of right atrial MAP in* patient group without known episodes of atrial fibrillation. ○ = values from patients who relapsed to atrial fibrillation within three months. ● = values from patients who maintained sinus rhythm for at least three months. □ = result of analysis of right atrial MAP during sinus rhythm in patient with spontaneously reverting attack of atrial fibrillation, followed by several episodes of irregular heart rhythm during the consecutive weeks. In two patients the mean values are indicated (large circles) as well as individual values of analyses of three different recordings (small circles).

sinus rhythm has been due to extracellular electrolyte abnormality.

Digitalization can convert atrial fibrillation to sinus rhythm and inadequate digitalis treatment might therefore be a reason for failure of attempted DC conversion of atrial fibrillation. This might be the mechanism in patients 27 and 50. Patient 27 had misunderstood the instructions and was not treated with digitalis before the DC conversion attempt. However, during the 14 days he was in sinus rhythm he was also treated with digitalis in an adequate dose. Patient 50 had a rather high heart rate during the few minutes he was in sinus rhythm and could possibly have been under-digitalized.

Possible electrophysiological mechanisms

The electrophysiological background to atrial fibrillation has been the subject of differing opinions. Hecht et al. (7) summarized that focal discharge or re-entry phenomena might be the underlying cause of atrial fibrillation. These mechanisms were

extensively discussed by Katz and Pick (12) who also pointed out that both mechanisms might be present at the same time during atrial fibrillation. The multifactorial cause of atrial fibrillation has been well described by Hoffman and Cranefield (8), Watanabe and Dreyfus (22) and Hecht (6). Sound electrophysiological reasoning" (6) rules out the alternatives of focal discharge or re-entry as the only factors responsible for the fibrillation.

The duration of a MAP is a good approximation of the duration of an action potential recorded from the same tissue with microelectrodes (10). As there is a relationship between the duration of the action potential and the refractory period (9), the investigation has revealed that a short atrial refractory period is present in some patients failing to maintain sinus rhythm after DC conversion of atrial fibrillation. A short refractory period favours the development of an arrhythmia due to the possibilities of an earlier excitation (22, 23). Thus the arrhythmia tendency in the present cases can at least partly be explained by this mechanism which hitherto has not been shown to occur in the intact human heart.

The present MAP recording technique (16) records the sum of the electric phenomena from a large number of cells. Thus within this cell group, different durations of the action potentials from single cells can exist without being noticed. In fact different muscle fibers of the cat atria have different durations of the action potentials (2). Irregular repolarization predisposes to arrhythmias due to the fact that potential differences between adjacent muscle fibers might initiate a new depolarization (6 8 22). In the two patients from whom more than one recording was obtained, the variation of the duration of the right atrial MAP was greater than has been observed in an arrhythmia-free patient group (17). The variability of the duration of the MAP might thus —in addition to the short duration of the MAP— be a factor predisposing to arrhythmias in the patients investigated.

Shortening of atrial action potentials occurs with decreased cycle length (9), acetylcholine or vagal discharge (19). In addition in vitro experiments have revealed that low extracellular sodium or high extracellular potassium shortens ventricular action potentials (1). Cycle length alterations, low extracellular sodium and high extracellular potassium concentration can be excluded as reasons for the shortened MAP duration observed. Excessive vagal discharge would have led to bradycardia as well and can probably also be ruled out as a reason for the short MAP duration.

The drugs used for premedication and anesthesia are not known to cause changes in the duration of atrial action potentials. Most likely these drugs are not responsible for shortening of MAP in the present study All patients received the same premedication and anesthesia except for case 23 Case 23 reverted to sinus rhythm spontaneously and was thus not in need of the drugs. Nevertheless the MAP was of abnormally short duration in this case.

The action of digitalis upon the duration of the atrial MAP is complex however no shortening of the atrial MAP by digitalis was apparent in a previous study (17) Digitalis can thus also be ruled out as a probable reason for the observed right atrial MAP shortening.

The DC shock itself can be blamed as the cause of the MAP shortening. This is contradicted by the finding in case 23 who reverted spontaneously to sinus rhythm and still had a short MAP In addition, the QT-time, which is correlated to the duration of ventricular MAP (18) has not been reported to be shortened after DC conversion of atrial fibrillation (13 24). The fibrillation episode might also be blamed for causing cellular metabolic disturbances which might lead to decreased MAP duration. Studies on potassium exchange in atrial fibrillation indicate that the fibrillation episode may contribute to a potassium loss from the atrial muscle (11) There are no direct studies of the effect of intracellular potassium depletion on the duration of an atrial action potential. However a shortening of ventricular action potential induced by ouabain was shown to be accompanied by potassium loss from the cell (15).

Ebert (5) reported that the potassium content in atrial specimens obtained during heart operations was lower in those patients who postoperatively developed atrial fibrillation compared to those who maintained sinus rhythm. However no recordings of action potentials from these tissue specimens were reported.

The reports discussed (5 11 15) suggest that the observed shortening of the right atrial MAP might be due to a low potassium content of the

atrial muscle in the patients who have failed to maintain sinus rhythm for 3 months.

The observation of Carmeliet (3) that a potassium-deficient atrial muscle specimen can exhibit spontaneous phase-4-depolarization has not been verified in the present investigation. Phase-4-depolarization might of course, in addition to the short duration of the atrial MAP and the exaggerated differences between the durations of atrial MAP from different recording positions, be a possible component of the multifactorial mechanism provoking atrial fibrillation.

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IMPAIRMENT OF GLOMERULAR FILTRATION IN FASTING FOR OBESITY

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Abstract. When obese subjects are subjected to therapeutic fasts there is always a small increment of serum creatinine. In ten patients studied, the average creatinine value was 0.94 ± 0.17 mg% before fasting and 1.35 ± 0.18 (mean $\pm$ S.D.) during fasting ($p < 0.001$). In seven patients endogenous creatinine clearance was lowered from 137 ± 33 ml/min to 87 ± 18 ml/min by fasting ($p < 0.01$). Inulin clearance values gave similar clearance values. Para-aminohippuric acid (PAH) clearance was studied in five subjects and was found to be unaffected by fasting. Renal function was restored rapidly by refeeding. We have at present no explanation for the described phenomenon.

Starvation has become a standard method for weight reduction in severely obese patients when caloric restriction and other measures have failed. The first clinical report on this method was published by Bloom (2), but fasting was, of course, practised already in biblical times. Since 1959 the tendency has been to increase the periods of complete starvation from originally a couple of weeks to as long as 249 days (13) numerous reports have shown that most individuals tolerate even prolonged fasting well. Drastic though it may seem, weight reduction by fasting is the quickest method to reduce excessive overweight, and the follow-up studies hitherto reported show that the results are at least as good as the long-time results seen with dietary methods (2, 5 14).

Therapeutic starvation is not free from hazards. Liver function is impaired temporarily during fasts. In some patients serum glutamic-oxaloacetic (S-GOT) and glutamic-pyruvic transaminases (S-GPT) and bilirubin are raised (14), and bromsulphtalein retention is increased (10). Serum uric acid is always elevated during fasts, but gout is only rarely precipitated (4). Some cases of unexpected sudden death have occurred (12) and the cause of death has not been completely understood.

Most of the weight loss during starvation is water and protein (1), which adds to the criticism of therapeutic fasts. However in many cases of obesity the rapid weight loss produced by prolonged fasts is a way to break the vicious circle of caloric overconsumption and to establish sounder eating habits.

We have used fasts ranging from 10 to 50 days in severly obese patients who all have been in one way or another handicapped by their obesity. On the whole the patients have been satisfied with the treatment. In the first patients whom we subjected to therapeutic starvation we observed increments of serum creatinine. This caused us to check glomerular filtration before, during and after fasting. We have recently reported in a preliminary communication (6) that fasting seems to lower the glomerular filtration rate temporarily. This paper gives the results of a study in ten obese patients subjected to therapeutic starvation.

MATERIAL AND METHODS

Ten obese patients, five men and five women ranging in age from 28 to 69 years, were subjected to starvation during 10 to 50 days. Table I presents the patients, the treatment and some clinical findings.

Three patients (nos. 2, 5 and 6) fasted for two different periods. These patients and two others (nos. 1 and 3) were also treated with ketogenic diet. The ketogenic diet furnished 900 calories, 85% as fat. All the patients except one (no. 9) were initially and after fasting treated with low calorie diet (800–1 000 calories). During starvation all patients developed ketoacidosis, as measured by urinary ketone bodies, but only five of the ten patients had their standard bicarbonate values lowered below 20 mEq/l. As seen in the last column in Table I, the uric acid level during starvation was raised in all patients. The serum

Table I. *Data on patients subjected to the study*

keto = ketogenic diet

Pat. no	Sex	Age (y)	Height (cm)	Initial body weight (kg)	Estimated over weight[a] (kg)	Average daily weight loss during starvation (kg)	Treatment
1	♂	60	167	89.5	22	0.56	1000 cal 10 d; fasting 10 d; 1000 cal 5 d; keto 10 d
2	♀	44	158	107.5	49	0.50; 0.35	800 cal 9 d; fasting 20 d; 800 cal 10 d; keto 10 d; fasting 15 d; keto 11 d
3	♀	62	158	92.7	35	0.54	1000 cal 10 d; fasting 10 d; 1000 cal 5 d; keto 5 d; 800 cal 25 d
4	♂	41	171	103.8	33	0.40	1000 cal 12 d; fasting 28 d; 1000 cal 11 d
5	♂	62	174	105.9	32	0.54; 0.58	1000 cal 15 d; fasting 15 d; keto 10 d; fasting 10 d; 1000 cal 9 d
6	♀	39	162	97.7	37	0.40; 0.27	1000 cal 10 d; fasting 22 d; keto 10 d; fasting 21 d; 800 cal 8 d
7	♀	55	160	88.9	29	0.69	1000 cal 10 d; fasting 10 d; 1000 cal 10 d
8	♂	69	172	100.0	28	0.56	1000 cal 15 d; fasting 15 d; 800 cal 7 d
9	♀	[illegible]	175	126.1	51	0.56	Isocaloric diet 2 000 cal 17 d; fasting 50 d
10	♂	44	183	115.2	32	0.55	800 cal 29 d; fasting 10 d; 800 cal 10 d

[a] Estimated by the formula: height (cm)–weight (kg)–100.

urea value was followed in eight patients. The difference between serum urea on admission and the lowest value observed during starvation is given in Table I, column 9. The average daily weight loss in the ten patients during the first period of starvation varied between 0.40 and 0.69 kg. The daily weight reduction in three patients during second starvation period varied between 0.27 and 0.58 kg.

Oral sodium (3–4 g/day) and potassium chloride (1.5–3 g) were given to patients 6, 7 and 9. Patients 1, 2 and 3 received only sodium chloride (1.5–3 g) and patient 10 only potassium chloride (2 g). Patients 4, 5 and 8 were not given salt. Fluid intake was in no case less than 1/day. Multivitamin tablets were given.

Creatinine clearance was calculated from two serum creatinine values taken at 8 a.m. and 4 p.m. respectively and the urinary creatinine excretion during 24 hours. Inulin clearance was determined according to Heyrovsky (8) and PAH clearance as described by Brun (3).

RESULTS

Serum creatinine

Table II presents the serum creatinine values in the ten patients before, during and after the various treatment periods. A highly significant increase was noted *during fasting* as compared to free diet before fasting ($p < 0.001$). Refeeding after fasting brought the serum creatinine value back to the starting value ($p < 0.001$). *Ketogenic diet* also seemed to raise the creatinine. An almost significant increase ($p < 0.02$) from the pretreatment values was observed in the five patients treated with ketogenic diet. The mean serum creatinine value *on low calorie diet* (1.01 ± 0.21) was slightly but not significantly higher than the mean value on free diet before treatment.

Glomerular filtration

Creatinine clearance was studied in seven of the ten patients (Table III and Fig. 1). It is obvious from this study that the increment of serum creatinine is caused by a lowered creatinine clearance. The mean creatinine clearance value before fasting, 137 ± 33 ml/min, differed significantly ($p < 0.01$) from the corresponding value

Drop in serum urea (mg%)	Lowest value of standard bicarbonate during starvation (mEq/l)	Maximal serum uric acid during starvation (mg%)
	21	10.0
33	8	10.5
	19	11.2
18	21	12.0
22	20	13.0
19	16	10.0
24	18	8.5
20	23	8.9
25	18	11.5
30	24	17.8

during fasting, 87 ± 18 ml/min. Inulin clearance, which was studied in patients 4, 5, 8 and 9, gave similar values for the glomerular filtration rate (GFR) as endogenous creatinine clearance except during refeeding, when inulin clearance for some reason seemed to be lower than creatinine clearance (Fig. 2). On the average, fasting caused a drop in GFR by 37 %.

PAH clearance, which was recorded in five patients, was not affected by starvation (Fig. 1 and Table III).

Creatinine clearance was only studied in two of the five patients on ketogenic diet (nos. 5 and 6) and this treatment was interposed between two fasting periods. The data are too few to permit any conclusions to be drawn about the effect of this diet on renal function, but serum creatinine remains elevated with this diet (Table II), indicating an impaired GFR. One patient (no. 6) showed her lowest GFR while on this diet (Fig. 1).

DISCUSSION

In the first patients subjected to therapeutic starvation we observed small but significant increments of serum creatinine. In at least one paper on therapeutic fasting this could be observed in the results (11), but was not commented on. We have observed that urinary excretion tends to diminish in starvation. This phenomenon has also been reported recently by Cahill et al. (9). Thus there ought to have occurred a drop in glomerular filtration. This study shows that overweight patients treated with starvation develop a lowered glomerular filtration, a disturbance that disappears on refeeding. None of the patients showed any clinical signs of renal insufficiency and serum urea values were always lower during than before fasting. The lowering of urea is logical and

Table II. *Serum creatinine during various treatments for obesity*

The mean value for each period is given in mg %. The number of observations is given in parentheses

Pat. no.	Free diet before treatment	800–1000 cal diet before fasting	During fasting	Ketogenic diet	After refeeding
1	1.10 (1)	1.30 (4)	1.60 (2)	1.25 (2)	1.20 (1)
2	0.90 (2)	0.90 (1)	1.43 (3)	1.23 (3)	0.80 (1)
3	1.00 (1)	0.93 (3)	1.10 (2)	1.00 (1)	1.00 (1)
4	1.30 (1)	1.15 (4)	1.33 (6)		0.80 (1)
5	0.90 (1)	1.23 (4)	1.55 (2)	1.23 (4)	1.00 (1)
6	0.70 (1)	0.70 (3)	1.20 (5)	1.15 (2)	0.80 (1)
7	0.90 (1)	0.75 (2)	1.30 (2)		0.80 (1)
8	0.80 (1)	1.10 (1)	1.30 (4)		1.00 (1)
9	0.85 (2)		1.14 (11)		
10	0.90 (1)	1.05 (2)	1.56 (8)		1.15 (4)
Mean ± S.D.	0.94 ± 0.17	1.01 ± 0.21	1.35 ± 0.18	1.17 ± 0.10	0.95 ± 0.16

Table III *GFR and PAH clearances before and during starvation (mean ± S.D)*

	GFR ($n=7$) (ml/min)		PAH ($n=5$) (ml/min)
Before fasting	137±33	$p<0.01$	492±120
During fasting	87±18		481±152

demonstrates the diminution of protein catabolism following protein malnutrition.

While therapeutic fasts produce a substantial yet transitory drop in GFR, PAH clearance is mainly unaffected, which confirms a recent report (9) that renal blood flow remains unaffected in starvation.

We have as yet no explanation for the lowering of glomerular filtration by starvation. Sodium and/or potassium depletion, ketoacidosis, decreased renal filtration pressure, diminished blood volume and/or extracellular volume might cause it. Some of our patients were given salt supplements, others not. Salt intake did not seem to influence the degree of GFR decrease. Quantitative determination of ketone bodies was not done, so we cannot correlate the impairment of GFR to the degree of ketoacidosis. Since renal blood flow estimated by PAH clearance, was unchanged, it is unlikely that a decreased renal filtration pressure or diminished blood volume caused the drop in GFR. Furthermore, we have in some cases determined blood volume, hematocrit, blood pressure and exchangeable sodium and potassium before and during total fasting, but this has hitherto not revealed the underlying mechanism. More advanced studies of renal physiology in these patients are difficult to motivate from an ethical point of view

The lowered GFR during starvation does not rule out fasts as a method for weight reduction in severe obesity At present, however the method should be used with caution or avoided in patients with signs of renal function impairment.

The clinical significance of our findings in a

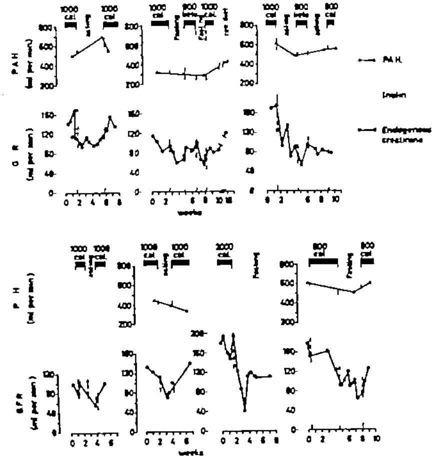


Fig. 1 Effects on renal function of therapeutic starvation.

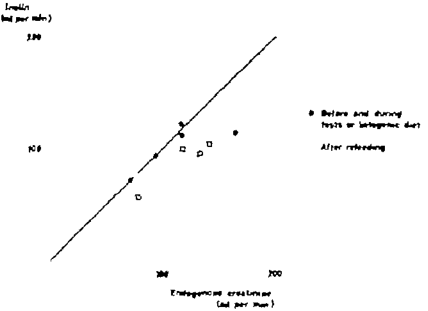


Fig. 2. Correlation between inulin and endogenous creatinine clearances.

wider sense is, of course, difficult to assess at this stage. It might be worthwhile to look at glomerular filtration in types of starvation other than therapeutic fasts for obesity. We know little about the renal reactions to starvation in people who are of normal weight or underweight, for instance, factors that may have importance for the care of the postoperative patient, the malnourished, and others.

Total starvation and ketogenic diet lower GFR significantly. There is, however, a tendency for GFR to decrease even on simple caloric restriction, even if the latter effect was not statistically significant in this study. This observation may be of importance in connection with the evaluation of renal function impairment by serum creatinine and creatinine clearance studies. Thus, when determining serum creatinine and endogenous creatinine clearance, the patient's diet should be taken into consideration.

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RENAL CLEARANCES OF DIFFERENT AMINO ACIDS IN WILSON'S DISEASE BEFORE AND AFTER TREATMENT WITH PENICILLAMINE

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Abstract. Two siblings with Wilson's disease are presented. They displayed no neurological symptoms and only minor hepatic dysfunction. In both patients signs of decreased tubular reabsorption of phosphate and urate were found. Measurements of clearances of the different single amino acids revealed initially high values for some of them, and later a definite and uniform decrease after treatment with penicillamine.

Patients with Wilson's disease are in a positive copper balance. It is assumed that the excess copper is in principle accumulated in the liver and only when this organ is saturated does it spill over to other organs such as the brain, giving rise to the descriptive name: hepatolenticular degeneration. Copper deposits have also been observed in limbus corneae as Kayser-Fleischer rings and in the kidneys, where anatomical studies point to pathological lesions predominantly in the tubules; the kidney glomeruli seem relatively unaffected (2, 15, 22).

Previous studies of the kidney function in patients with Wilson's disease have often demonstrated signs of tubular disorders, such as decreased reabsorption of uric acid (3, 13), phosphate (1), amino acids and glucose (4) and decreased acidification of urine (8, 20) in the presence of normal or slightly decreased glomerular function (1, 11).

Increased urinary excretion of amino acids in patients with Wilson's disease has been noticed since 1948 (19). Quantitative studies of single amino acid clearances in such patients do not seem to exist in the literature, and there is some uncertainty as to the reversibility of the condition after treatment. Further it is not known whether the potential nephrotoxicity of penicillamine (7, 21) also includes damage to the reabsorptive capacity of tubules for amino acids.

In this paper we present two cases of Wilson's disease in which clearance of several amino acids has been carefully determined prior to treatment, after 5 and 11 months treatment, respectively with D-penicillamine (β_1 β-dimethylcysteine).

METHODS

Renal function was evaluated by determination of glomerular filtration rate, effective renal plasma flow, clearance of amino acids and urate, and tubular reabsorption of phosphate.

For determination of the glomerular filtration rate the classic clearance technique was used. Oral fluid intake was initiated two hours before the beginning of the clearance period. Blood and urine samples for determination of blank values were taken half-an-hour before the start of the clearance period. Sodium iothalamate tagged with ^{125}I was used. Twenty to 25 μCi was added to 500 ml isotonic NaCl. This solution was divided into priming and sustaining doses, so that a plasma activity of 800 to 1 800 counts/min/ml (cpm) was obtained. For determination of effective renal plasma flow ^{131}I-hippuran was used. Twenty microcuries were divided into priming and sustaining dose. The priming dose was added to 5 ml isotonic NaCl and the sustaining dose added to 500 ml of isotonic NaCl. A plasma activity of 500 to 800 counts/min ml (cpm) was obtained. Activity was measured in a gammacounter equipped with a dual-channel pulse height analyzer to a statistical inaccuracy of less than 1%. Clearances were calculated according to the formula $C = U \cdot V/P$ where C is clearance in ml/min, U the concentration in the urine in cpm, V the urine volume in ml/min and P the concentration in the plasma in cpm. Clearances were expressed as the average of two or three periods of about 30 min, the separate clearance determinations giving closely related results.

The free amino acids of plasma and urine were determined by ion exchange chromatography using gradient elution technique with sodium-citrate buffers. Taurine,

Table I *Clearances of single amino acids in case 1 Measurements performed before treatment and 5 and 11 months after treatment with penicillamine*

	Clearances (ml/min/1 73 m² BSA)		
	Before treatment	5 mo. after	11 mo. after
Taurine[a]	6.4	3.0	9 4
Aspartic acid	2.7	3.2	1.5
Threonine	1.5	0.6	0.7
Serine	2.8[b]	1.0[b]	1.9
Glutamine + asparagine			0.4
Glutamine acid[c]	0 1	0.8	0.3
Proline	0.0	0.0	0.04
Glycine	6.7	2.1	2.3
Alanine	0.7	0.4	0.2
½ cystine cysteine			0.4
Valine	4.5	0 6	0.4
Methionine	0.5	0 5	0.7
Isoleucine	0 5	0 6	0.1
Leucine	0.7	0.5	0.3
Tyrosine	1.0	0.8	1 0
Phenylalanine	1.0	0.8	0.9
Lysine	0.5	0 4	0.3
Histidine	26.5	3.1	2.9
Arginine	0.4	0.3	0.0

[a] Uncertainty about the true levels of taurine in plasma (12).
[b] Serine + glutamine + asparagine.
[c] Questionable.

aspartic acid, threonine serine and glutamine + asparagine were determined on Beckman Amino Acid Analyzer B at 30°C and the remaining acids on Beckman Analyzer C at 54°C Blood plasma was deproteinized by precipitation with picric acid and the excess of picric acid in the filtrate removed by Dowex 2 X8 in the chloride form as described by Stein and Moore (18). The plasma samples from the last determinations after 11 months treatment were deproteinized immediately after centrifugation of the blood The other samples of plasma were deproteinized af ter standing for some weeks in deep-freezer which prevents the possibility for a quantitative determination of cystine in the protein-free filtrate (5). The analyzed urines were freed from the major part of their ammonia content.

Clearance of urate and phosphate-reabsorption were determined on the basis of 24-hour urinary excretions and mostly averaged from several determinations.

Serum copper was determined according to a method described by Gubler et al. (10), and modification hereof was applied for determination of urinary copper.

Urate in serum and urine was determined by the uricase method described by Praetorius (9).

CASE REPORTS

Case 1

♀ 17 years. The patient was admitted due to haemolytic episodes but exhibited in addition biochemical signs of parenchymatous liver disease. The suspected diagnosis of Wilson's disease was confirmed by findings of low content of coeruloplasmine and copper in serum and high urinary excretion of copper. A liver biopsy disclosed severe degeneration of cells and heavy deposits of copper. However, the amount of connective tissue was only slightly increased. Clinical examination showed no neurological disorders. A barely visible Kayser-Fleischer ring was found in limbus corneae by slit-lamp examination.

Kidney glomerular filtration rate and effective renal plasma flow were normal or only slightly decreased, but some signs of tubular dysfunction were found. Clearance of urate was increased and phosphate reabsorption decreased. There was no glycosuria, but high clearances of glycine, valine and histidine were seen (Table I). The levels of the different amino acids in plasma were normal.

Treatment with penicillamine induced an instantaneous cessation of the haemolytic episodes. Through the following months an improved synthesis of albumin in the liver was indicated by a rising albumin level in blood. At the same time the serum urate level increased and urate clearance normalized (Fig. 1 Table II).

Plasma amino acid levels remained normal, but the amino acid clearances decreased, now indicating a completely normal tubular reabsorption in the kidneys (Table I).

A repeated liver biopsy after one year of treatment revealed a normal structure of the liver cells, though copper deposits were still considerable.

The patient remained in good general condition throughout the treatment period. No side-effects to penicillamine treatment were seen.

Case 2

♂ 14 years. Brother of case 1 Investigation of the family of case 1 disclosed that sister had previously died at the

Table II *Clearances of urate iothalamate (= inulin clearance) and para-amino-hippuric acid and tubular reabsorption of phosphate in both patients before and after treatment with penicillamine*

	C-urate (ml/min 1 73 m² BSA)	T bular reabs. PO_4(%)	C_{iothal} (ml/min/ 1 73 m² BSA)	C_{PAH} (ml/min/ 1 73 m² BSA)
Case 1				
Before treatment	27	76	69	321
After one year treatment	5.8	86	88	350
Case 2				
Before treatment	23	82	78	394
After one year's treatment	9 4	90	79	360
Normal range	6–12	> 85	60–120	375–600

age of 12 years from a liver cirrhosis of unkown origin. This brother presented macroscopically visible Kayser Fleischer ring, and diagnosis of Wilson disease was confirmed by finding low copper and coeruloplasmine content in serum and high urinary excretion of copper.

In this patient no biochemical signs of liver disease were seen. A biopsy showed, however, severe cellular degeneration and extensive deposits of copper. No signs of cirrhosis were observed.

The kidney glomerular filtration rate and effective renal plasma flow were normal. Tubular kidney function was impaired as indicated by an elevated urate clearance and high clearances of threonine, glycine, alanine, tyrosine and histidine (Table III). Phosphate reabsorption was decreased. No glycosuria was found.

After treatment with penicillamine, liver synthesis of albumin deteriorated temporarily but was normal again *after one year (Fig. 2). A repeated liver biopsy at that* time showed complete normalization of the cell structure in spite of persisting massive deposits of copper.

During treatment the urate clearance and the elevated amino acid clearances decreased. Phosphate reabsorption increased (Fig. 2, Tables II and III). The patient was permanently perfectly well and showed no clinical signs of disease except for the Kayser-Fleischer ring in cornea.

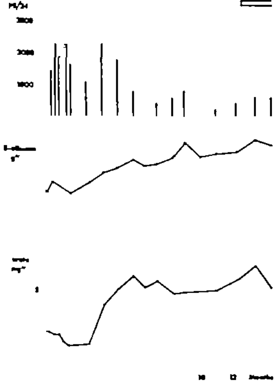


Fig. 1 Urinary excretion of copper serum albumin and serum urate in case 1 before and during treatment with penicillamine. *Note the fall of urinary excretion of copper* to normal values when penicillamine was discontinued for few days after one year's treatment.

Table III. *Clearances of single amino acids in case 2. Measurements performed before treatment and 5 and 11 months after treatment with penicillamine*

	Clearances (ml/min/1 73 m² BSA)		
	Before treatment	5 mo. after	11 mo. after
Taurine[a]	11.5	9 6	15.8
Aspartic acid	3.8	4.3	3.4
Threonine	6.7	1.8	1.0
Serine	5.8[b]	2.6	3.6
Glutamine + asparagine			1.0
Glutamic acid[c]	1.6	0 8	0.8
Proline	0.0	0.0	0.0
Glycine	16 4	7.9	7 1
Alanine	2 8	1.0	0 8
½ cystine + cysteine			0.5
Valine	0.5	0.5	0.5
Methionine	1 4	0 8	1.0
Isoleucine	0.5	0 4	0 4
Leucine	0 6	0 5	0 5
Tyrosine	3.8	2.2	2.3
Phenylalanine	1.6	1 7	1.6
Lysine	0 6	0 9	0.5
Histidine	20 7	16.7	13.3
Arginine	0.5	0 3	0.3

Uncertainty about the true levels of taurine in plasma (12).
Serine + glutamine + asparagine.
Questionable.

DISCUSSION

Renal tubular dysfunction in patients with Wilson's disease is usually considered of minor importance compared to the hepatic or cerebral lesion, although rickets has been described as the initial feature (14)

The effect of penicillamine treatment on neurological and hepatic symptoms is well documented. The same statement is not true as to the effect on renal tubular dysfunction. Since the introduction of penicillamine treatment in 1956 only few reports concerning the *reversibility* of the tubular phenomena have appeared in the literature. Walshe (20) and Fulop et al. (8) observed disappearance of glucosuria after treatment of a single patient in three years and one year respectively Fulop et al. also reported signs of improved reabsorption of phosphate and urate after one year's treatment of the same patient. Both authors investigated urinary acidification in several patients. Fulop et al. found defective acidification in 4 of 12 patients despite 1 $/_2$–9 $/_2$ years' penicillamine treatment. Walshe found in a prospective study that the acidification defect largely disappeared in eight patients after one years' treatment.

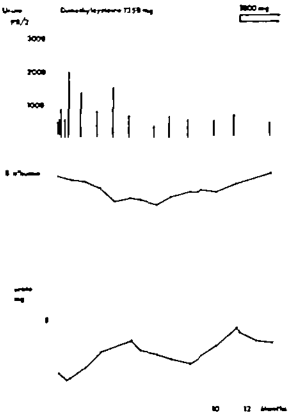


Fig. 2 Urinary excretion of copper serum albumin and serum rate in case 2 before and during treatment with penicillamine.

Aminoaciduria unaffected by penicillamine treatment has been described in one patient by Morgan (14) and in one patient by Walshe (20) Walshe (20), Fulop et al. (8) and Falkmer et al. (6), however noticed diminished aminoaciduria in a few other patients after treatment. The authors of the present article have not been able to find studies in the literature concerning renal clearances of single amino acids in Wilson's disease or of the influence of penicillamine treatment hereupon.

Normal values of amino acid clearances have not been mentioned in the present article, since earlier reports have shown wide variations (16)

Studies of single amino acid clearances in the two cases presented in this paper disclosed no specific pattern, which is in accordance with previous investigations of urinary excretion of amino acids. Remarkably high clearances of threonine, glycine, alanine, valine, tyrosine and histidine have been observed in one or both patients before treatment. High excretion of these amino acids has been reported earlier e.g. threonine (4 14 17), glycine (14 17) alanine (14) valine (14 17), tyrosine (14 17), histidine (14) High excretions of other amino acids have also been reported earlier (4 14 17).

We find it of interest that all the strikingly high clearances of single amino acids in both our patients decreased significantly during treatment with penicillamine. The effect in these two patients was most remarkable during the first six months of treatment. More severely affected patients may require a longer period of treatment before an amelioration of the tubular function is seen. Still it may not be impossible for an irreversible damage to occur after a longer duration of the disease.

It is conceivable that numerical expressions of renal tubular function, such as clearances of phosphate, urate and single amino acids, may add information concerning the effectiveness of penicillamine treatment. An improvement of the tubular reabsorptive capacity probably means that copper is being removed from the tissues.

Treatment with penicillamine has not, in the two cases presented, given rise to proteinuria or aminoaciduria.

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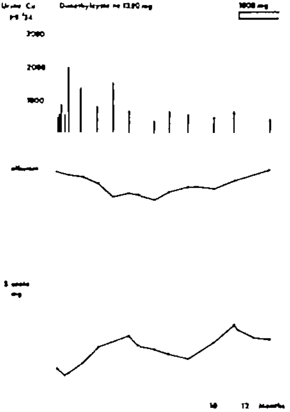


Fig Urinary excretion of copper serum albumin and serum urate in case 2 before and during treatment with penicillamine.

Aminoaciduria unaffected by penicillamine treatment has been described in one patient by Morgan (14) and in one patient by Walshe (20). Walshe (20) Fulop et al. (8) and Falkmer et al. (6), however noticed diminished aminoaciduria *in a few other patients after treatment*. The authors of the present article have not been able to find studies in the literature concerning renal clearances of single amino acids in Wilson's disease or of the influence of penicillamine treatment hereupon.

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DISTRIBUTION OF SERUM LIPIDS IN NORWEGIAN RECRUITS

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Abstract. 324 young men attending medical examination for military service had their sera tested for cholesterol and triglycerides. The subjects were grouped according to physical fitness, relative weight, smoking habits, blood pressure and ischaemic heart disease among relatives. Serum triglyceride values were found correlated to relative weight, smoking habits and ischaemic heart disease among relatives. A similar but less obvious, trend was observed in serum cholesterol.

In the last 15 years a large number of population studies have been carried out in order to assess what should be accepted as normal serum lipid values (1 2, 4 5 10 12, 13 15, 20, 22, 25 28 30, 33 35 36). There are however few extensive studies dealing with serum lipids in young adults (2, 25 27 30, 36). Several factors are known to influence serum lipid levels, i.e. age, sex, heredity smoking habits, physical fitness, climate, ethnic differences, nutritional status (12, 33). Through their influence on the serum lipid pattern all of these factors have been incriminated in some way in the pathogenesis of atherosclerosis and ischaemic heart disease (IHD).

From an epidemiological point of view attempts to correlate these risk factors to serum lipid patterns at an age prior to the development of clinical manifest atherosclerosis should be of importance, especially if a close follow-up could be performed.

This study was undertaken to evaluate serum lipid patterns among healthy young adult males and to correlate these values to factors known to influence serum lipid levels.

MATERIAL AND METHODS

The material consisted of 324 young males attending medical examination for military service in May–June 1969. They were all born in 1950 and all living in western Norway. No attempt at selection was made.

A questionnary was filled in by each subject. Attention was paid especially to smoking habits, physical activities and occurrence of IHD among relatives. The questionnary for smoking habits was filled in as follows:

1. Non-smoker (or stopped smoking more than year ago).
2. Smoker (or stopped smoking less than year ago. These were all classified as 2 *a*, regardless of previous smoking habits.) (*a*) Light smoker = less than 50 cigarettes/week. (*b*) Moderate smoker = 50–100 cigarettes/week. (*c*) Heavy smoker = more than 100 cigarettes/week. Pipe-smokers, 15 in all, were classified according to cigarette equivalents consumed (one pipe was presumed to equal one cigarette).

Physical activities were classified as follows:

1. No training.
2. Regular training (regardless of frequency). Only physical activities and training at leisure were registered, with no regard to physical activities at work.

Ischaemic heart disease was defined as:

1. Typical angina d'effort relieved by nitroglycerine.
2. Sustained myocardial infarction.

As siblings were included, parents, grandparents, sisters and brothers of parents. The grouping was:

1. No IHD among relatives.
2. IHD among relatives, diagnosed before the age of 65 years.

The medical examination followed a special questionary including physical examination, with registration of height, weight, blood pressure (BP), pulse and urine analyses (for glucose and protein, using Uristix, Ames).

Only subjects judged healthy (from questionary and physical examination) and fit for military service were included in the study.

A weight/height index was calculated from the formula (29) *weight 100/((height 0.71) − 53.9)*, and the subjects were grouped according to relative weight as follows:

1. < 80 %
2. 80 – 89 %
3. 90 – 110 %
4. 111 – 120 %
5. > 120 %

Table I. *Distribution of serum lipids in the different subgroups*

Group		Cholesterol			Triglycerides		
		Mean	S.D	$p^a <$	Mean	S.D	$p^a <$
Training	100	170.0	29.4	—	60.7	25.5	0.05
No training	224	175.8	31.4	—	67.5	30.9	—
Smoker	167	173.9	30.9	—	67.8	31.1	—
Non-smoker	157	174.2	30.8	—	62.9	27.6	—
Without IHD among relatives	291	173.6	30.7	—	64.1	29.6	0.02
IHD among relatives	33	177.9	31.7	—	76.7	27.1	—
Relative weight (%)							
Below 80	19	157.1	26.3	—	49.8	20.9	—
80–89	107	172.0	31.3	0.05	61.6	26.6	—
90–110	185	174.6	30.0	0.02	67.0	27.9	0.01
111 120	7	197.1	19.8	0.01	87.7	40.6	0.01
Abov 120	6	213.8	25.2	0.01	109.5	68.6	0.01
Test group I, score 1 3 (6 subjects not tested)	32	166.9	32.4	—	73.5	34.2	—
Test group II score 4–6	197	173.6	30.9	—	64.8	29.8	—
Test group III score 7–9	89	177.3	29.7	—	64.0	27.1	—
Systolic BP (mmHg)							
Below 110	32	166.9	26.1	—	73.5	28.8	—
110–140	201	173.7	32.5	—	64.6	29.5	—
Above 140	91	177.2	28.3	—	64.4	29.7	—

^{a} p 0.05 not registered.

Blood pressure was measured with the subjects in sitting position. Two measurements ere made, one at the beginning and one t the end of examination. The mean was recorded. The diastolic BP was recorded at the disappearance of the 5th Korotkow sound. No correction was made for arm circumference (32). The grouping was established somewhat arbitrarily

1 Systolic BP (*a*) 110 mmHg, (*b*) 110–140 mmHg, (*c*) >140 mmHg.
2. Diastolic BP (*a*) <90 mmHg, (*b*) 90–100 mmHg, ( ) >100 mmHg.

T judge physical fitness, an exercise test was performed on a bicycle ergometer and oxygen uptake was calculated. The results were graded 1–9 physical fitness paralleling an increasing score:

1 O uptake <30 ml/kg/min
_ O uptake 31 34 ml/kg/min
3. O uptake 35–37 ml/kg/min
4. O_2 uptake 38–41 ml/kg/min
5 O_2 uptake 42–45 ml/kg/min
6. O uptake 46–49 ml/kg/min
7 O_2 uptake 50–53 ml/kg/min
8. O_2 uptake 54–59 ml/kg/min
9. O uptake >60 ml/kg/min

As overnight fast could not be secured in all subjects, the blood samples were drawn in the afternoon, at least 8 h postprandially

Lipid analyses

Total cholesterol was estimated by the ferric chloride method of Klungsøyr et al. (26) and triglycerides were analysed according to Dumbach (17). The precision of the methods was calculated from 58 replicate analyses of the same serum according to the formula.

$$\pm 2\sqrt{\frac{\Sigma(d^2)}{N}}$$

where d = difference between duplicates, and N = total number of determinations. "95 limits": cholesterol ±7%, triglycerides ±12.5%.

All data were coded and transferred to punched cards. The calculations were performed by an electronic computer

Statistical analyses

Mean, standard deviation (in skewed distributions not identical with that in normal distributions) (6), coefficient of correlation and test of skewness, were calculated according to standard formulas (8). In evaluating differences between averages (Student's *t*-test, strictly valid only in normal distributions) (18), the modified formula proposed by Kemp and Nielsen (23) was applied.

RESULTS

The results are presented in Table I and Figs. 1–3 For all subjects tested the following mean values were found. Serum cholesterol 174 mg% (S.D 30 mg%) Serum triglyceride 65 mg% (S.D 29 mg%) The frequency distributions of values for serum cholesterol and serum triglyceride concentrations in all subjects are given in Figs. 1 and _.

The distribution of triglycerides is rather asymmetric, and clearly skewed to the right (coefficient of skewness = 1.45) The cholesterol values show a more symmetric distribution (coefficient of skewness = 0.55). A vague correlation is found between concentration of serum triglycerides and serum cholesterol (Fig. 3 coefficient of correlation = 0.37)

Subgroups

Subjects in training seemed to have lower triglyceride values than subjects not in training ($p < 0.05$). No differences in cholesterol values were found (Table I).

A similar trend is observed between smokers and non-smokers: slightly lower triglyceride values among non-smokers, not significant, and no differences in serum cholesterol values. No differences are observed in lipid values between light, moderate and heavy smokers.

In the subgroups, with and without IHD among relatives, the findings are more clear-cut, with elevated triglyceride values among subjects with IHD among relatives ($p < 0.02$) and no differences in cholesterol values.

Taking height/weight indices, we find increasing cholesterol and triglyceride values paralleling relative weight.

Subjects with relative weight above 110% have significantly elevated triglyceride and cholesterol values compared to normal (relative weight 90–110%) and lean subjects (relative weight below 90%). The findings in the different fitted groups are inconclusive.

Comparing subjects with systolic BP above 140 mmHg and subjects with systolic BP below 110 mmHg, a trend to slightly elevated cholesterol values is observed in the former group. No changes are observed in diastolic BP (One subject had diastolic BP above 100 mmHg. His lipid values were: cholesterol 205 mg% triglyceride 110 mg%.)

A group was extracted with the following characteristics:

1. Training.
2. No IHD among relatives.
3. Non-smoker
4. BP < 130/90
5. Relative weight: 90–110%

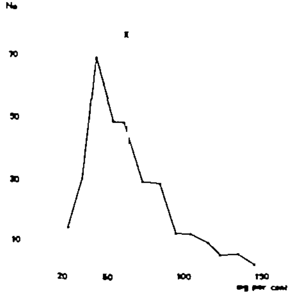


Fig. 1 Frequency distribution of triglycerides. Class interval = 20 mg %. $\bar{X}$ = mean. Six subjects had triglyceride values above 150 mg%

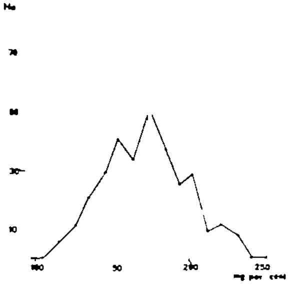


Fig. 2. Frequency distribution of cholesterol. Class interval = 20 mg %. $\bar{X}$ = mean. T subjects had cholesterol values abov 260 mg%

Eleven subjects fulfilled the criteria, with the following lipid values: Mean cholesterol 159 mg% mean triglycerides 54 mg% and all subjects within 30–70 mg% triglycerides and within 120–199 mg% cholesterol.

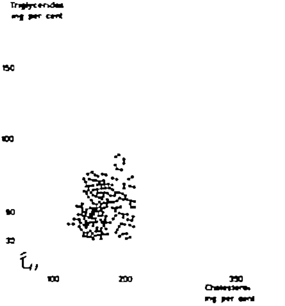


Fig. 3 Relationship between serum cholesterol and serum triglyceride values.

Table II *Cholesterol values reported in literature*

Literature	Age (y.)		Values in mg %	Comments
Adlersberg et al. (1)	18–22	22	185 2 ± 11 76	
Albrink et al. (2)	20–29	59	201	No S.D mentioned
Carlson et al. (12)	15–19	3	152.7 ± 5.8	
Hayes et al (20)	20–29	13	206	
Keys et al. (24)	17 20	343	173.6 ± 31.2	
Konttinen (27)	18–25	314	202.3–213 1	
Lewis et al. (28)	20–24	42	200.6	
Nicolaysen (30)	20–21	102	157	Blood donors
Nicolaysen (30)	20– 1	17	198	Telegraph employee
Nicolaysen (30)	19–21	36	153 ± 28.8	Medical students
Schaefer (35)	20–32	34	212 (137 548)	
Svanborg et al. (36)	16–35	62	191.5 ± 7.3	

DISCUSSION

The results of this study should be taken as values obtained from a healthy population.

Our results seem to agree fairly well with those from similar studies (Tables II and III) Assaying cholesterol by the method of Klungsøyr et al. (26) is known to give results on the average 14% higher than those obtained by Schoenheimer Sperry's method. Correcting for this methodological difference, the mean serum cholesterol value in our study is 149 ± 25 mg% clearly less than reported by most workers cited in Table II but in close approximation to that of Carlson et al (12). Comparing the method of Klungsøyr et al. with that of Sperry Webb, no significant difference could be found (Closs: personal communication) As regards triglycerides, most workers seem to agree on the serum level in healthy young adult males. 80 ± 30 mg% Our results are 65 ± 29 mg% Using tripalmitin as standard our results in mmol/l are 0 81 ± 0.37 in good agreement with the findings of Carlson et al. (Table III)

A skewness in distribution of triglycerides has been observed by others (11 31 35) In this study the skewness is most obvious among lean (relative weight below 80%) and does not exist among obese subjects. Similarly most workers find cholesterol more symmetrically distributed (25) This is, however a more controversial finding (12, 28).

The influence of physical activity on serum triglyceride values is well documented (12, 21) Physical activity and serum cholesterol values are more controversial, and so far no clear picture is encountered (19 24 34) Our results—no significant differences among subjects in training/not in training—could perhaps be explained by the normal activity of life among healthy males 19 years of age. Their activity at work and off work, at all levels higher than in later years, might outweigh the influence of supplementary training.

An association between smoking and serum lipids has been noted in several epidemiological studies. Elevated serum cholesterol values have been observed among smokers by some (7 9 12) but not by others (27) Regarding triglycerides and smoking, epidermiological studies are scanty Carlson et al. (12) found higher triglyceride values in smoking than in non-smoking men up to 55 years. From the present study no clear-cut picture is encountered Possibly the period of smoking might be too short (eight years at most, averaging two to three years) to have any influence on the serum lipid levels.

Among the subjects with and without IHD among relatives the results are difficult to evaluate. As the age of parents and relatives ranges from 40–90 years, the population at risk varies, partly caused by age (IHD above 65 years was not registered) and partly by other factors (number of relatives, sex, other diseases, stature smoking habits, nutrition etc.) Other pitfalls are: IHD among relatives was evaluated from a questionary without any examination of them. Rela-

Table III *Triglyceride values reported in literature*

Literature	Age (y)		V lues	Comments
Antonis et al. (5)	30	26	80 ± 21 mg/ 100 ml	Bantu male
	30	16	86 ± 26 mg/ 100 ml	European male
Carlson et al. (12)	15–19	3	0.80 ± 0 06 mmol/l	
Hayes et al. (20)	20–29	13	2.79 mEq/l	No S D mentioned
Schaefer (35)	20–32	34	82 (32–852) mg 100 ml	
Svanborg et al. (36)	16–35	30	83 5 ± 34 4 mg 100 ml	

tives classified as not suffering from IHD might well have advanced atheromatosis without symptom of disease. The subjects questioned might be insufficiently informed concerning the health of their relatives.

Deutscher et al. (14), in a comprehensive study demonstrated a close correlation between serum cholesterol values among children and their fathers and mothers. Thomas (37) found increased serum cholesterol values among offspring whose parents were afflicted with IHD No such correlation could be found in this study possibly because the trend is too scarce to be detected in only a few samples ($n = 32$)

An association between serum triglyceride values in the normal range (< 150 mg %) and IHD *among relatives has never been reported. Further* studies are required to substantiate our findings, together with a close follow-up of the afflicted subjects.

The opinion as regards serum lipids and body weight is rather controversial, partly due to methodological difficulties: how to measure body fatness? Either by height/weight measurements, or by some somatological index? Most workers seem to agree on a correlation between serum triglycerides and body weight (3 4 12) As regards cholesterol there is no such unanimity (12, 24 28) In the papers cited, lipid values among normal and over-weight subjects were assessed without further grouping of relative weight and serum lipids. A more differentiated grouping, together with a more homogenous material, could perhaps explain our findings.

Papers concerning the relation, if any between hypertension and serum lipids are scanty and no clear-cut picture has emerged (4) Our results warrant no definite conclusion about serum lipids and BP

Comprehensive studies (1 4 14 16, 21 30, 38, 39) have disclosed a relationship between IHD and physical activity smoking, heredity high BP and obesity perhaps mediated through their common influence on the serum lipid levels. From an epidemiological point of view when these risk factors could be ruled out, serum lipid levels might be considered the ideal or optimal in the age group concerned (33) i.e. among healthy young adult males in the postabsorptive state, the ideal lipid values should be: cholesterol 120–200 mg % triglycerides 30–70 mg %

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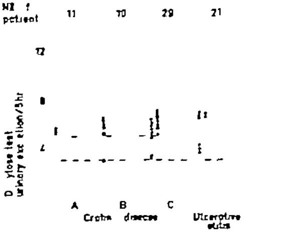


Fig. 1. D-xylose test in 50 patients with Crohn disease and 21 patients with ulcerative colitis. ○ = patients with earlier small bowel resections, ● = patients with no resections.

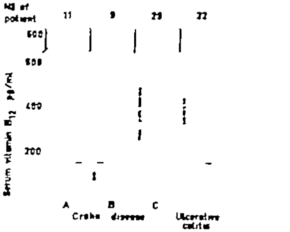


Fig. 3. Serum vitamin B_{12} in 49 patients with Crohn's disease and 22 patients with ulcerative colitis. Symbols as in Fig. 1.

Rice (9) as modified by Kerstell (8). Excretion of >5.0 g xylose during 5 h was considered as normal, 3–5 g as borderline values and <3.0 g as abnormal.

Fat excretion was determined when the patients were kept on diet containing 100 g fat daily. Faeces were collected in one or two 3-day periods. The faecal fat was determined according to van de Kamer et al. (7). Faecal fat excretion <18 g/72 h was considered as normal, 18–30 g as borderline values, and values >30 g/72 h as abnormal.

Vitamin B_{12} in serum was determined by the microbiological method of Hunter et al. (5) using Euglena Gracilis (Z-strain). Vitamin B_{12} < 150 pg/ml was regarded as abnormal.

Schilling test without intrinsic factor was carried out as described by Gräsbeck et al. (3). Urinary excretion of radioactive vitamin B_{12} in 48-hour collection below 5% was considered abnormal and 5–10% excretion as borderline value. In patients with abnormal or borderline Schilling test gastric acid secretion was studied and defective intrinsic factor secretion could be excluded.

Folate in serum and whole blood was assayed with Lactobacillus casei according to the technique described by Hansen (4). Values of serum folate <3.0 ng/ml and whole blood folate <35 ng/ml were considered as abnormal.

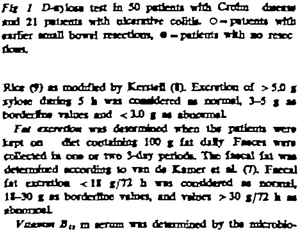


Fig. 2. Faecal fat in 41 patients with Crohn's disease and 18 patients with ulcerative colitis. Symbols as in Fig. 1.

RESULTS

D-xylose absorption. Of the fifty patients with Chron's disease only one, belonging to group A, had an abnormally low D-xylose value (Fig. 1). Borderline values were seen in 4 out of 11 patients in group A, in 1 out of 10 in group B, and in 8 out of 29 patients in group C. In 21 patients with ulcerative colitis borderline values were found in 4.

Faecal fat. In all patients with Crohn's disease belonging to groups A and B the faecal fat excretion exceeded 18 g/72 h and in two-thirds of them values more than 30 g/h were noted (Fig. 2). These patients, with long history of Crohn's disease and previous extensive small bowel resections, had values of 183, 263 and 331 g/72 h. Both in group C and in patients with ulcerative colitis about 50% of the patients had an excretion above 18 g/72 h and 25% had values above 30 g/72 h.

Vitamin B_{12} in serum. About one-fourth of the patients with Crohn's disease belonging to groups A and B had vitamin B_{12} values in serum below

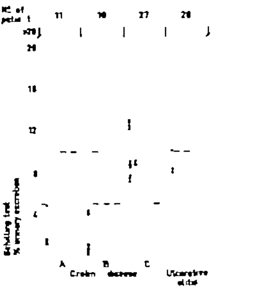


Fig. 4. Schilling test in 48 patients with Crohn's disease and 20 patients with ulcerative colitis. Symbols as in Fig. 1.

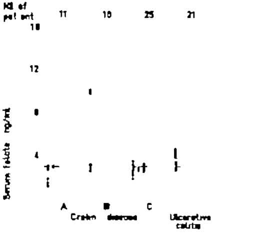


Fig. 5. Serum folate in 46 patients with Crohn's disease and 21 patients with ulcerative colitis. Symbols as in Fig. 1.

150 pg/ml, and these had all been operated upon with small bowel resection (Fig. 3). In group C one-tenth of the patients had low concentration of serum vitamin B_{12}. *The patients with ulcerative* colitis had normal vitamin B_{12} values.

Schilling test. A decreased vitamin B_{12} absorption was common in patients with Crohn's disease (Fig. 4). In groups A and B 50% had Schilling test values below 5% and the remaining patients in these groups had borderline values, except for 3 patients out of 11 in group A who had normal values. The 7 patients with values below 2% were all operated upon with resection of the small bowel. In group C more than half of the patients had borderline values and only one out of 27 had less than 5% excretion. In the patients with ulcerative colitis 40% had borderline values. No abnormally low values were seen among the 20 patients in this group.

Folate in serum and whole blood. About half of the patients with Crohn's disease had serum folate values below 3 ng/ml (Fig. 5). The frequency of low values was similar in the three groups. About one-tenth of the patients had whole blood folate values below 35 ng/ml (Fig. 6). In ulcerative colitis one-fourth of the patients had serum folate below 3 ng/ml, but whole blood folate concentration was normal in all instances.

DISCUSSION

In the present study the *D-xylose* test revealed subnormal values in 14 out of 52 patients with Crohn's disease. There was, however, no correlation between the extension and localization of the lesions and the abnormal values. A low *D*-xylose value was noted frequently, not only in patients with extensive involvement of the small intestine but also in patients with involvement only of distal ileum and/or colon. The one markedly low value was found in a patient with moderate extension of the lesion in the jejunum. Also in some patients with ulcerative colitis subnormal values were found. It thus seems that the *D*-xylose test poorly

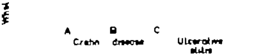


Fig. 6. Whole blood folate in 46 patients with Crohn's disease and 22 patients with ulcerative colitis. Symbols as in Fig. 1.

reflects the localization and extension of Crohn's disease in the intestine and, therefore, is of minor value for clinical evaluation of the disease. Nor does this test contribute to the differentiation of Crohn's disease from ulcerative colitis

It is obvious from the present study that malabsorption of *fat* is frequently encountered in Crohn's disease In all patients with more extensive small bowel involvement impaired fat absorption was observed. Also in about half of the patients with only minor involvement of the small intestine and in about half of the patients with ulcerative colitis the faecal fat excretion was increased.

In Crohn's disease a derangement of the vitamin B_{12} absorption is frequently seen. However an abnormally low absorption (Schilling test below 5%) was with few exceptions seen only in patients who previously had undergone small bowel resection. None of the patients with ulcerative colitis had a Schilling test below 5% but border line values were seen almost as frequently as in the patients with Crohn's disease who had only moderate involvement of the ileum and/or colon. It is questionable whether a moderately reduced vitamin B_{12} absorption is of clinical significance. Only a few of the patients with borderline values in the Schilling test had low concentration of vitamin B_{12} in serum. In most of these patients small bowel resection had been performed earlier This finding strongly supports the view (2) that patients with Crohn's disease who have undergone small bowel resection should be substituted with vitamin B_{12}. In ulcerative colitis no vitamin B_{12} deficiency was observed.

It has been impossible to settle whether the patients had received folate prior to the investigation. Consequently no comparison between the groups concerning the frequency of low values can be made. Low serum folate concentrations were, however frequently seen in Crohn's disease and the whole blood folate concentration was low in 6 patients. At least in these 6 patients a defective folic acid absorption has to be suspected (4).

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ANTAGONISM BETWEEN GLUCOSE AND EPINEPHRINE REGARDING INSULIN SECRETION

A Dose-Response Study in Man

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Abstract. A dose-response study of the inhibitory effect of epinephrine on glucose-induced insulin secretion in man has been performed. The results demonstrate the exquisite sensitivity of the pancreatic β-cells to epinephrine, as small a dose as 3 $m\mu g$/kg/min inducing 50% inhibition. These studies emphasize the physiological significance of epinephrine for the regulation of insulin secretion. They also show that in previous studies in man and animals supramaximal amounts of epinephrine have been employed. Our results also suggest that the actions of epinephrine and glucose on insulin secretion are competitive. Infusion of aminophylline had only a minor inhibitory effect on the action of epinephrine. Therefore, our data do not permit the conclusion that epinephrine suppresses insulin release exclusively by diminishing the rate of synthesis of cyclic AMP. Other possible mechanisms are discussed.

We have previously suggested that glucose exerts its insulinogenic action through a direct stimulation of a specific membrane receptor which stimulates adenyl cyclase (9). The activation of this enzyme leads to an increased rate of formation of cyclic adenosine 3',5'-monophosphate (cyclic AMP), this nucleotide then inducing the release of insulin. The postulated glucose receptor is closely related to, or has characteristics similar to, those of the β-adrenergic receptor since the β-adrenergic blocking agent propranolol inhibits glucose-induced insulin secretion (6). According to this description, glucose may be regarded as a β-agonistic substance in the pancreas.

The pancreatic β-cell is also equipped with α-adrenergic receptors which, on stimulation, inhibit insulin release. This phenomenon has largely been studied by the use of catecholamines in vivo (17, 22, 23, 24, 25) and in vitro (10, 11, 18, 19, 20). Thus, epinephrine has been well documented as a *powerful inhibitor of insulin release*. Provided glucose acts as a β-agonist, the degree of inhibition of insulin secretion by the α-agonist epinephrine would depend not only on the dose of epinephrine but also on the concentration of glucose. The experiments presented in this paper favour this view.

MATERIAL AND METHODS

Fifty-two studies were performed on eight healthy non-obese, non-hospitalized volunteers, two women and six men, aged 24–51 years, with normal intravenous glucose tolerance. Their insulin response to glucose infusion was considered normal according to our standards (7).

The studies were done in the morning with the subjects fasting since the previous evening. Indwelling needles were placed in a brachial vein of each arm and kept patent with slow saline infusions. One was then used for the infusion of glucose and the other for blood sampling.

The glucose infusion test (GIT) was performed according to Cerasi and Luft (7). An initial rapid injection of 500 mg of glucose/kg b.wt. was followed by the infusion of [illegible]0 mg of glucose/kg/min for 60 min. Blood samples for the determination of glucose and insulin were taken before and 5, 10, 20, 30, 40, 50, and 60 min after the start of the glucose injection.

In the epinephrine–GIT experiments, the catecholamine infusion was started 30 min before the GIT and continued throughout the experiment. Epinephrine was administered at a constant rate in the doses 3, 10, 20, 60 and 90 $m\mu g$/kg b.wt./min. Blood samples for glucose and insulin determinations were collected at −30, −20, −10 and 0 min and thereafter as above.

In another series of experiments the effect of aminophylline on insulin release during the administration of epinephrine and glucose was studied. Aminophylline was given as a priming injection of 200 mg followed by the infusion of another 200 mg during the 60 min preceding

Table I. *Effect of epinephrine infusion on the initial (0–30 min) and later (30–60 min) insulin responses to glucose infusion (insulinogenic index)*

Upper values, epinephrine infusion, lower values, infusion of epinephrine plus aminophylline

Subject no.	Control		Epinephrine infusion (mμg/kg/min) 90		60		30		10		3	
	0–30	30–60	0–30	30–60	0–30	30–60′	0–30′	30–60′	0–30′	30–60	0–30′	30–60′
1	40.34	56.03	2.65	7 15	3 78	11.83			18.04	30.16	42.25	52.22
					10.50	7 17			28.41	38.86	52.22	56.04
2	53 46	60 61			4.51	5 40	8.28	10.45	11.26	11.86	16 15	15.88
					4.52	5.08	8.69	9.60	18 08	19 12	36.56	22.53
3	24.37	28.47			0.31	2.22	3.10	8.42	17.82	17.52	9.38	11.54
					2 44	4 09	3.67	7.59	9 00	12.49	5.54	12.31
4	11.63	7.68	2.19	2.90	2.99	3.50						
5	15.24	20 79	0 08	1.85								
6	16.10	17 54	1.22	4 10			6.40	12.77				
							3.97	12 15				
7	31 13	37 46	0.49	2.35	0.57	2.71	1.57	3 49	6.12	8.79	9 76	11.99
					0.33	1 73	5.91	10 13	4.93	10.68	7.25	13.28
8	33.35	22.75	10.90	5.21	0.63	3 74			11.58	9.15	24 87	15.35
									26.13	26.58	26.75	23.02

the GIT The experimental conditions were otherwise as in the epinephrine–GIT experiments with the addition of blood samples taken at −60, −55 and −45 min.

At least one week was allowed to elapse between the studies in the same subject.

Blood glucose concentration was measured enzymatically with commercial glucose oxidase preparation (Kabi Reagens, Stockholm), insulin by the double antibody method (13)

The epinephrine and aminophylline used in this study were Adrenalin (ACO) and Teofyllamin (ACO), both standard drugs manufactured in Sweden. T the epinephrine solutions was added 1 0–1.5 mg/ml of ascorbic acid as an antioxidant.

The insulin response to glucose is expressed as the ratio between the area beneath the glucose curve with the base at 0 mg/100 ml and the area under the insulin curve with the base t the 0 min insulin value (insulinogenic index). The insulinogenic index for the 0–30 min areas represents mainly the initial insulin response, while the 30–60 min index represents the later response. The values presented are insulinogenic index 100.

RESULTS

A synopsis of the results is presented in Table I. Table II summarizes the effect of different doses of epinephrine on the insulin response to glucose, and Table III the influence of aminophylline on these effects.

It is apparent from Table II that epinephrine in all doses used here markedly inhibited both the initial and later insulin responses to glucose, the paired differences being significant in all instances. The individual sensitivity to epinephrine showed marked differences, however This was most obvious in the lower dose ranges of epinephrine

Table II. *Effect of epinephrine on the insulinogenic index*

Epinephrine dose (mμg/kg/min)	N of experi-ments	0–30 min Control	Epinephrine	Mean difference ±S.E.M.	30–60 min Control	Epinephrine	Mean difference ±S.E.M.
90	6	24 6±4 8	2 9±1 7	21 7±4.38[a]	27 1±7 0	3 9±0 8	23 12±6.55[c]
60	6	32.4±5.8	2.1±0.8	30.3±5.48[a]	35.5±8.3	4.9±1.5	30.59±7.45[b]
30	4	31.2±8 0	5.9±1.1	25.3±7.39[d]	36 0±9.2	10.5±0.9	25 6±9 45[d]
10	5	36 3±5 1	13 0±2.3	23.5±5.67[c]	41 1±7.5	15.5±4.0	25 6±6.72[c]
3	5	36.5±5.0	20.5±6.1	16 0±6.56[d]	41.1±7.5	21 4±7.8	19.6±7.22[d]

[a] $p<0.0025$. [b] $p<0.005$. [c] $p<0.01$ [d] $p<0.05$.

ions from the dose–response relationship presented in Fig. 1 in the present work that 90 mμg is already a supramaximal dose. Indeed, our study demonstrates the exquisite sensitivity of the human pancreatic β-cells to epinephrine. With a dose as low as 3 mμg/kg/min, 50% of the maximal inhibitory effect of epinephrine on insulin release could be demonstrated. Taking into consideration the short half-life of catecholamines ($2^1/_2$ min) (30) and a normal concentration of catecholamines in plasma of 300–400 mμg/l (15, 16) it is obvious that the administration of 3 mμg of epinephrine/kg/min may be considered to be within the framework of a physiological experimental model.

The inhibition of insulin release by epinephrine was obvious in the initial as well as later phase of the insulin response to glucose infusion. Tables I and II and Fig. 1 show that the magnitude of this inhibition was similar for both phases. However, as can be seen in Fig. 2, the plasma insulin values reached near the end of the glucose infusion were considerably higher than during its early phase, especially with the higher doses of epinephrine. Furthermore, the blood glucose levels at the end of the glucose infusion were much higher than during its initial period. This implies that the inhibition of insulin release induced by epinephrine can be overcome by increasing the stimulus to insulin release, glucose. This is also evident from the fact that, when using the highest epinephrine concentrations, the sudden increase in blood glucose level induced by the priming injection of glucose could elicit a significant initial insulin response. This occurred even when the basal insulin level was depressed by epinephrine.

Porte has demonstrated convincingly that epinephrine inhibits insulin secretion by stimulating the α-adrenergic receptors of the pancreatic islets (22). The intracellular mechanism involved in the mediation of this inhibition from the α-receptor to the locus of insulin release is unknown, however Hellman et al. (14) have presented evidence for the thesis that epinephrine inhibits insulin release by suppressing the utilization of glucose in the β-cell. The concentration of epinephrine used in their experiments, however, is of such a magnitude (2 000 mμg/ml) that the physiological significance of their findings is questionable. Indeed, Ashcroft et al. (1) using much lower concentrations of epinephrine (200 mμg/ml)—concentrations still far beyond the physiological range—were not able to demonstrate any interference of epinephrine with the glucose metabolism of the β-cell. In our studies, a concentration of 3 mμg of epinephrine/kg/min, although very efficient in inhibiting insulin release, was without effect on the basal blood glucose level. This may indicate that such low doses of epinephrine have no demonstrable effect on glycogenolysis and peripheral glucose utilization.

It has been well established that stimulation of the β-adrenergic receptors by epinephrine results in an increased rate of formation of cyclic AMP (27). On the other hand, contradictory data exist regarding the effect of α-adrenergic stimulation on cyclic AMP synthesis. Turtle and Kipnis (28) demonstrated that the increase in cyclic AMP obtained in isolated pancreatic islets with theophylline could be inhibited in the presence of a massive dose of epinephrine (10 000 mμg/ml). The α-adrenergic blocker phentolamine, could reverse this inhibition. These studies indicate that the locus of the inhibitory action of epinephrine is adenyl cyclase. Since cyclic AMP is involved in insulin release (18, 21, 29), inhibition of the production of this nucleotide would easily explain the suppression of insulin secretion by epinephrine. Studies on the effect of α- and β-adrenergic stimulation on adipose tissue adenyl cyclase seem to favour this hypothesis (4).

More recent studies have suggested another mechanism for epinephrine inhibition of insulin release. Feldman and Lebovitz (1[illegible]) obtained inhibition with epinephrine in vitro not only of glucose-induced insulin release but also of insulin secretion caused by exogenous cyclic AMP (dibutyryl cyclic AMP). They concluded that epinephrine, via stimulation of α-adrenergic receptors, acts on a locus beyond the formation of cyclic AMP, tentatively at the site where cyclic AMP exerts its effect.

In this connection we should mention the recent studies in humans by Sutherland's group (2), demonstrating an increase in cyclic guanosine 3′ 5′-monophosphate (cyclic GMP) in blood following the infusion of epinephrine together with β-adrenergic blockers. Thus it does not seem impossible that cyclic GMP induced by stimulation of the α-adrenergic receptors might inhibit competitively the intracellular action of cyclic AMP induced by β-adrenergic stimulation.

It is obvious from the pertinent literature that the mechanisms by which epinephrine inhibits insulin release are far from clear. We are of the opinion that the main reason for this is the use of huge concentrations of the different agents as well as the lack of careful dose-response studies.

In the present study we have attempted to influence the intracellular concentration of cyclic AMP by the addition of aminophylline to all doses of epinephrine used. We have previously demonstrated that the present dose of aminophylline did not significantly modify the insulin response to glucose infusion in healthy subjects (8). On the other hand, aminophylline infusion was effective in restoring the suppressed insulin release induced by the β-adrenergic blocker propranolol (6). This was interpreted as showing that this concentration of aminophylline was effective only when the rate of formation of cyclic AMP was reduced. According to Table III and Fig. 1 there seemed to be a tendency for aminophylline to diminish the inhibitory action of epinephrine. This effect, however, was insignificant except for the later insulin response to glucose at the lowest dose of epinephrine. This negative result makes it less probable that epinephrine inhibits insulin release exclusively by diminishing the formation of cyclic AMP.

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REPEAT HEMODYNAMIC STUDIES IN PATIENTS WITH ARTERIAL HYPERTENSION TREATED MEDICALLY FOR SEVERAL YEARS

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Abstract. Twenty-three patients with arterial hypertension were studied before treatment and after 2 to 7 years on routine antihypertensive regimens with saluretics, alone or in combination with various hypotensive agents. Systemic hemodynamics at rest and during standardized exercise load in the sitting position on a bicycle ergometer were obtained in all subjects. At the initial study there were no significant hemodynamic differences between 14 adequately treated patients (group A), i.e. casual outpatient diastolic blood pressures generally ≤100 mmHg, and 9 inadequately treated patients (group I). At the repeat study after treatment, the brachial artery blood pressure and systemic vascular resistance were significantly lower both at rest and during exercise in group A compared to the other group, while there were still no significant differences in heart rate and cardiac output. The lowered mean brachial artery blood pressure in group A was mainly due to a significantly decreased cardiac output both at rest and during exercise, and the reduction in systemic vascular resistance was not statistically significant. Contrary to the adequately treated patients in group A, two untreated patients had conspicuously higher values for mean brachial artery blood pressure and systemic vascular resistance when restudied after an interval of 5 years. It is concluded that an adequate and vigorous long-term treatment of arterial hypertension will benefit the circulation and decrease cardiac load. The need of search for therapies with more physiological effects is pointed out.

A primary goal of active antihypertensive treatment in human arterial hypertension should be to restore, as permanently as possible, the hemodynamic pattern to that of normotensive subjects. This should preferably be maintained not only under resting conditions, but also under different forms of mental and physical strain. Whether the generally accepted and clinically used antihypertensive regimens are capable of achieving this in long-term, sustained treatment of arterial hypertension has so far been little studied and reported. This is easily understandable as studies of this kind are associated with considerable practical and technical difficulties. The data reported are conflicting, probably due to the small and selected series studied. Thus some authors (1–3) report on a further increase in the systemic vascular resistance after long-term treatment, although both blood pressure and cardiac output were reduced. More favourable effects on the increased systemic vascular resistance with various hypotensive drugs administered over long periods have been reported by others (6, 7).

This communication presents our findings in 23 hypertensive patients, studied hemodynamically at rest and during standardized exercise both before and after active treatment for 2 to 7 years. For comparison the results from similar studies in 2 untreated patients are included.

MATERIAL AND METHODS

Follow-up studies after 2 to 7 years were completed in 25 patients with arterial hypertension. They were selected from a larger group of subjects with raised blood pressure whose hemodynamic pattern was studied during rest and exercise. For analyses of the results the 23 treated patients were referred to one of two subgroups: *Adequately treated patients (A)*, whose casual outpatient diastolic blood pressures under treatment generally were ≤100 mmHg. Fourteen patients were referred to this group. *Inadequately treated patients (I)*, with casual diastolic blood pressures generally >100 mmHg. This group numbered nine subjects.

Some pertinent clinical data are given in Table I. All cases but two were diagnosed as having essential hypertension; two patients in group A had renal hypertension. At the time of the first hemodynamic study no patient was under antihypertensive treatment. Fourteen patients had never, or not for several years, received hypotensive agents. In the remaining nine patients all such drugs were withdrawn before the first study. In eight cases 2 weeks

Table I. *Clinical data on patients in groups A and I*

	Group A	Group I	Untreated
No. of pats., sex	14 (9 ♂ 5 ♀)	9 (3 ♂ 6 ♀)	2 (1 ♂ 1 ♀)
Initial age (y)	48.8 ± S.E. 2.5	47.3 ± S.E. 2.9	52, 43
Duration of treatment (y)	4.3 ± S.E. 0.3	5.4 ± S.E. 0.3	5 5
Keith–Wagener grade (no. of pats.)			
Before 0	—	5	—
Before 1–2	13	3	2
Before 3–4	1	1	—
During 0	3	1	—
During 1–2	11	8	2
During 3–4	—	—	—
Initial plasma creatinine			
≤ 1.2 mg%	11	7	2
1.3–1.5 mg%	3	2	—
Weight (kg)			
Before	73.7 ± S.E. 3.1	71.7 ± S.E. 3.3	67 53
During	74.4 ± S.E. 3.0	72.1 ± S.E. 3.3	71 52

or more and in one patient receiving only pentolinium 3 days before the study. When first studied, no patient was in overt cardiac or renal failure or had cerebral symptoms. Several of them had unequivocal radiological and ECG signs of marked left ventricular hypertrophy and strain, and in five patients the plasma creatinine was slightly elevated to 1.3–1.5 mg%. All patients were informed about the purpose and method of the study and gave consent to its performance.

After thorough clinical work-up establishing the diagnosis and excluding curable forms of hypertension, therapy was instituted. The patients were then dismissed for further check-ups on an outpatient basis. All but three patients were seen regularly by us over the years. Individualized therapy was given aiming at an optimal effect on the BP with no or easily endured side effects. This meant some adjustment of doses of drugs, especially in the early part of the follow-up. The various types of antihypertensive drug regimens used appear in Table II. The basic therapy was in all cases a saluretic agent, usually chlorthalidone.

After 2 to 7 years of treatment the patients were admitted to the hospital for a second hemodynamic study and for general examination. The changes seen in Keith-Wagener grading and b.wt. since the first study are shown in Table I. No patient had clinically deteriorated as regards cerebral, cardiac or renal function. In contrast, several patients, especially in group A, presented with less marked changes in ECG and on chest X-ray. Serum creatinine was normalized in three patients. Small average increases in b.wt. were seen in both groups.

The two untreated patients were studied at an interval of 5 years. Up to the time of the second study there was no change in their initial Keith-Wagener grading or renal function.

The examination procedure for obtaining hemodynamic data, and the techniques used for their analysis have been described in detail elsewhere (8). Studies were carried out in the morning with the patients in fasting state and included the following: heart rate, intra-arterial blood pressure, cardiac output using a dye-dilution technique with bromsulphalein as indicator and with intermittent sampling of arterial blood, and oxygen consumption through collecting expired air in a Douglas bag for subsequent gas analyses according to the method of Scholander (9). From the data obtained, stroke volume in ml/beat and systemic vascular resistance in arbitrary units were calculated.

After percutaneous insertion of catheters under local anesthesia into a brachial artery and into the right atrium or subclavian vein, the patients rested comfortably sitting in an arm-chair for one half-hour before the first measurements were made. Another set of determinations was then obtained during physical effort. The patients performed standardized exercise load sitting on an electrically braked, variable-load bicycle ergometer (Elema-Schonander AB, Sweden). The cardiac output was measured during the last minute of the exercise period, which usually lasted for 10 min. The level of exercise was adapted to the subject's tolerance. In each patient the protocol at the repeat study was identical to that used at the first examination.

The Student *t*-test was used for testing differences between groups and paired differences within the groups.

RESULTS

The levels of the intra-arterial blood pressure before treatment at the initial study were nearly identical in the two groups of patients both at rest and during exercise (Figs. 1 and 2). In relation to the normal standards in this laboratory both groups were characterized by an elevated brachial artery blood pressure and by increased systemic vascular resistance, normal cardiac output, and heart rate in the upper normal range. There were initially no significant differences between groups A and I for these functions, neither at rest nor during exercise.

At the repeat study after several years treat

Table II. *Antihypertensive regimens used during the treatment*

Treatment regimen	Group A (*n*)	Group I (*n*)
Saluretics alone	5	2
Saluretics + methyldopa/reserpine	5	2
Saluretics + hydralazine	1	1
Saluretics + sympathetic inhibitors	2	3
Saluretics + sympathetic inhibitors + methyldopa/hydralazine	1	1

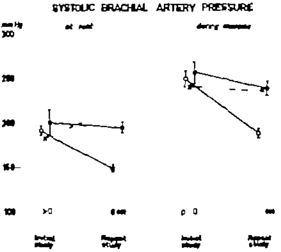


Fig. 1. Systolic brachial artery blood pressure before and after 2 to 7 years with or without antihypertensive treatment. The average values together with the standard error are indicated for group A (O) and group I (●), for the two untreated patients ( ) the average values only. The p-values refer to the significance levels of differences between the groups A and I.

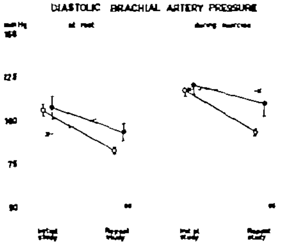


Fig. 2. Diastolic brachial artery blood pressure before and after 2 to 7 years with or without antihypertensive treatment. Symbols as in Fig. 1.

ment, differences appeared between the hemodynamic patterns of the two groups. As expected from the criteria for subdividing the patients, group A had almost normal systolic and diastolic brachial artery blood pressures at rest, and also during physical exercise the B.P. was kept at a normal level (Figs. 1 and 2). Patients in group I showed a lesser degree of B.P. reduction at rest, and during exercise the B.P. still reached high levels. Comparing the new B.P. levels obtained during treatment, group A had significantly lower systolic and diastolic brachial artery blood pressures than group I. This difference was maintained or accentuated during the exercise load. The average systolic and diastolic brachial artery blood pressures of the two untreated patients were distinctly higher at the second study at rest, while the exercise B.P. were apparently unchanged.

The heart rate, cardiac output and stroke volume, on the other hand, showed a general trend to lower average figures in all three groups. There

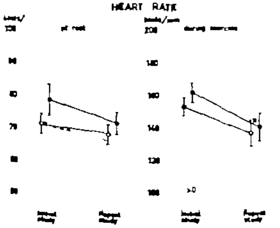


Fig. 3. Heart rate before and after 2 to 7 years with or without antihypertensive treatment. Symbols as in Fig. 1.

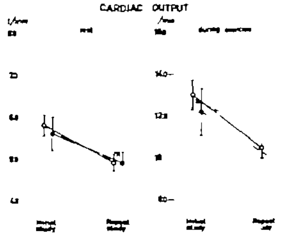


Fig. 4. Cardiac output before and after 2 to 7 years with or without antihypertensive treatment. Symbols as in Fig. 1.

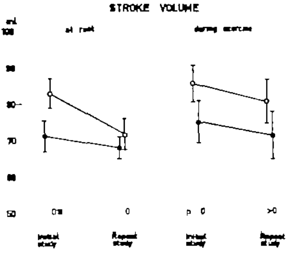


Fig. 5. Stroke volume before and after 2 to 7 years of antihypertensive treatment. Symbols as in Fig. 1.

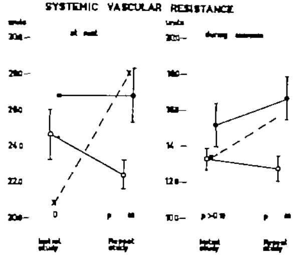


Fig. 6. Systemic vascular resistance before and after 2 to 7 years with or without antihypertensive treatment. Symbols as in Fig. 1.

were still no significant differences after treatment between groups A and I in these data, neither at rest nor during exercise (Figs. 3–5). Some changes were also noticed for the oxygen consumption, the average figures at rest decreasing from 0.26, 0,23 and 0.29 to 0.24 0.22 and 0.25 l/min for groups A and I, and untreated patients respectively. The corresponding figures for the exercise period were from 1.46, 1.16 and 1.37 to 1.42, 1.02 and 1.33 l/min respectively. At no stage was there any significant difference between the oxygen consumption in groups A and I.

The changes in systemic vascular resistance (Fig. 6) of the two groups were similar in distribution to those of the blood pressures. Thus the initial average figures, both at rest and during exercise, were statistically not different for groups A and I, but at the repeat study were significantly lower in group A compared to group I. The average resting figure for the calculated resistance in group I was completely unchanged after treatment. During exercise it was even higher than at the initial study thus accentuating the gap between the two groups. The two untreated patients increased their resistance both at rest and on exercise particularly the resting figures were higher.

Besides comparing the average actual levels of the various data, also the averages of paired differences have been compared (Fig. 7). The drop in mean brachial artery blood pressure in group A at rest was statistically significant, and the drop became even more marked during exercise. The lesser decrease in group I was of the same order both at rest and during exercise and only the exercise figure reached statistical significance.

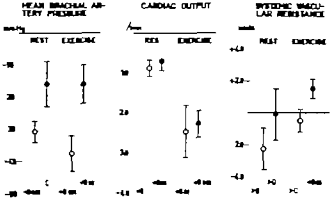


Fig. 7. Average differences together with standard error in mean brachial artery blood pressure, cardiac output and systemic vascular resistance after 2 to 7 years of antihypertensive treatment. The *p*-values refer to the significance levels of paired differences in the two groups. Symbols as in Fig. 1.

The decrease in cardiac output at rest was significant in both groups, and the reduction became considerably greater during exercise. Contrary to the B.P., the decreases in cardiac output were of the same order in groups A and I, both at rest and on exercise.

The systemic vascular resistance showed a small and insignificant decrease in group A at rest and during exercise. There was no change at all in the calculated resistance in group I at rest. During exercise a significant rise was observed.

DISCUSSION

These results indicate that common antihypertensive regimens, using saluretic agents as basic therapy will keep the blood pressure at a lower level during sustained treatment over several years, primarily through reducing the cardiac output, while the systemic vascular resistance is only moderately or not at all affected. This might be considered as little gratifying, as the logical aim for treatment of established arterial hypertension should be to normalize the blood pressure through reducing an increased systemic vascular resistance. From a clinical point of view however the main objective may be to lower the burden on the heart and to lessen the strain on the vessels, without unfavourably interfering with the blood supply to vital organs such as the brain and kidneys. These conditions may be reasonably met also through lowering the cardiac output, with regional variations in vascular resistance and redistribution of blood to vital areas. As a result, certain less vital areas, e.g. the striated muscles, may end up with a vascular resistance not only unchanged, but even higher compared to the initial state before treatment. Moreover if the theory presented by Brod (2) is valid, that untreated hypertensive patients have a low resistance of the vessels in the muscles, this will not deteriorate the metabolic processes of these muscles. A decrease in cardiac output at rest of the magnitude observed here may thus be of uncertain importance. The cardiac output decreases with advancing age (10), but the decrease found here may well be exaggerated. Patients with mitral stenosis may have low cardiac output without any signs of congestive heart failure, and without indication of malfunction in any organs (12). As long as the peripheral circulation can be regulated in a way to safeguard the circulation to vital organs at rest and to ensure a proper increase of blood flow during exercise, there is no reason to believe that hypoxemic changes may be caused by a decrease of total flow

The present investigation was carried out to analyse the hemodynamic effects of our rather diversified routine treatment of arterial hypertension, rather than to study the effects of any special antihypertensive drug or antihypertensive regimen. With the present results, however it is natural to question whether other types of drug regimens than those used would have resulted in different hemodynamic effects during prolonged treatment of arterial hypertension. There is no conclusive answer to this question. Reubi and Blütkofer (7), with a similar type of drug regimen as we with saluretics as basic therapy found a decrease in peripheral resistance that was largely responsible for the fall in B.P seen in 13 patients studied after 13 to 31 months of treatment. However only the two patients with the highest initial resistance values exhibited a substantial decrease, the other 11 showing varying results. Only cuff blood pressures were obtained. It is not clear whether the B.P readings used were obtained in direct connection with the cardiac output determination or not.

Findings similar to those of Reubi and Blütkofer have been reported by Johnson and Jones (5), who studied 6 patients after 2 to 13 months of treatment with saluretics in combination with guanethidine or reserpine. They also observed reductions in the exercise B.P especially in patients treated with guanethidine. Bello et al. (1) restudied 12 hypertensive patients after 9 to 17 months on therapies designed to reduce sympathetic function. They found, on the contrary the most consistent hemodynamic change associated with lowering of B.P to be a reduction in cardiac index, and an increased total peripheral resistance.

Lund-Johansen (6) restudied three groups of altogether 31 hypertensive men treated for 8 to 12 months with three different saluretics. A control group of 7 men was restudied after 8 to 12 months without treatment. The results indicated different modes of action for chlorthalidone and thiazides. The pressure drop in the thiazide group was usually associated with a reduced peripheral resistance, and in the chlorthalidone group with a reduction in cardiac index. However the patients in the latter group, compared with the other groups, were

older and thinner with arterial hypertension of considerably longer duration, and further studies may be needed before any definite conclusions can be drawn concerning variations in mode of action of saluretic drugs commonly used for chronic treatment of arterial hypertension.

Hydralazines may cause peripheral vasodilation with reflexly increased cardiac output during prolonged peroral administration. Two new substances recently introduced for the treatment of arterial hypertension have similar properties (4 11). It is possible that the incorporation of any of these, or of similarly acting drugs, in a combined treatment would give a more physiological result of long term treatment of raised B.P Troublesome side effects and the difficulty of using drugs necessitat ing administration several times daily have so far precluded this type of attack.

In group I the systemic vascular resistance at rest was unchanged at the repeat study after treat ment. This should be contrasted with the sharp rise of the vascular resistance in the untreated pa tients, even if there were only two of them. The systemic vascular resistance usually increases with increasing age (10). Thus, even the modest decrease in the brachial artery blood pressure recorded in group I may have been of benefit to the patients, and some of them also showed clinical improvement as judged from ECG or chest X ray examination.

The more pronounced drop in brachial artery blood pressure in group A compared to group I was achieved through a reduction in the systemic vascular resistance. This decrease of B.P into the normal range was maintained, and even accentuated, during physical exercise. It seems, there fore, that a more aggressive mode of treatment, forcing the B.P down toward the normal range, may yield such beneficial hemodynamic effects that such treatment should always be attempted. Only when disturbing side-effects appear or when inability of the patients to follow instructions is obvious, should less vigorous treatment be ac cepted.

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EXPERIENCE OF IMMUNOSUPPRESSIVE THERAPY OF GLOMERULAR DISEASE IN ADULTS

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Abstract. A report is given on 25 adult patients with glomerular disease treated with prednisolone and azathioprine for 3–35 months (mean 13 months). Renal biopsy has been obtained in all cases except one. In 12 patients significant improvement in inulin clearance and/or proteinuria has been recorded. Eight patients maintained a clinical steady state, while in five patients the disease progressed. The good results were obtained in patients with proliferative glomerulonephritis or minimal change glomerulonephritis and in one patient with lupus nephritis, but complete remission was not attained in any patient. The role of immunosuppressive treatment in acute glomerulonephritis is discussed, and the difficulties in assessing the therapeutic effect versus spontaneous improvement are stressed. The question is also raised whether an unchanged state or retarded progress in a downhill course may sometimes be regarded as a positive result of treatment.

During the last decade the use of immunosuppressive drugs in glomerulonephritis has become widespread. In addition to corticosteroids different cytostatics have been used and especially azathioprine. Good therapeutic results are often reported in children (1, 10), sometimes with steroids alone. The effects in adults have been variable, although disappointing results seem to predominate (1, 3, 12, 15). The main difficulty in evaluating the treatment is the variable natural course of glomerular disease. In view of this we find it valuable that results of clinical trials continue to be published in order to establish a more secure basis for evaluation of the therapeutical approach to glomerular disease in adults.

MATERIAL AND METHODS

The investigation included 25 patients with glomerular disease, 11 males and 14 females, aged 19–58 years. The criteria for subjection to the study were clinical and/or histological evidence of glomerulopathy. Diabetics and patients with immunosuppressive treatment for less than 3 months were excluded. Percutaneous renal biopsy was performed in all patients except one, and electron microscopy was available in six patients. Repeat biopsy was performed in only two patients. In one case normal kidney tissue was found on light microscopy, no electron microscopy being performed, and in one case the biopsy was not conclusive (Table I). Patients with mixed proliferative and membranous glomerulonephritis were referred to the proliferative group. Eight patients appeared with an acute poststreptococcal nephritis and 17 with an insidious onset. The patients have been grouped into those with a history of less than 6 months, from 6 months to 3 years, and those with a history of more than 3 years before the start of the immunosuppressive treatment (Table II). Nephrotic syndrome was diagnosed in 14 patients and five of them had a history of less than 6 months, one with a preceding throat infection, and the other four an insidious onset of the disease. Diastolic blood pressure of 100 mmHg or more (range 100–130 mmHg) before treatment was registered in nine patients, but the pressure was satisfactorily suppressed in all patients except one during the study. The roentgenologic kidney size was normal in 16 patients, increased in two nephrotic patients, and clearly reduced in two patients.

Two patients had been treated with steroids alone for 2 months and for 2 years, respectively, before azathioprine was added. Azathioprine was initially administered in a dosage of 2–3 mg/kg b.wt. and the maintenance dose was then adjusted individually according to the white blood cell count, usually within a range of 1.5–2.5 mg/kg b.wt. Prednisolone was given with individual variations, initially 60–100 mg/day for 2–4 weeks, then gradually reduced to a long-term maintenance dose of 5–10 mg/day after 3–4 months. The duration of treatment varied from 3 to 35 months with a mean duration of 13 months. At the end of the study 11 patients were still on treatment.

When evaluating the effect of the treatment we have used the following parameters: serum creatinine (determined with the Technicon Autoanalyzer), protein excretion and erythrocyte excretion at the regular control visits and clearances of inulin (7) and para-aminohippuric acid (4) at longer intervals. The conventional inulin clearance was in some patients replaced by a single injection inulin test (2). Owing to the difference in the follow-

Table I. *Response to immunosuppressive therapy in glomerular disease*

T = single injection inulin test (SIIT)

Case	Age	Sex	Histological classification	Duration in months: at onset	Duration in months: at therapy	Serum creatinine before therapy	Serum creatinine after therapy	Inulin clearance before therapy	Inulin clearance after therapy	PAH clearance before therapy	PAH clearance after therapy	Filtration fraction before therapy	Filtration fraction after therapy	Urinary protein before therapy	Urinary protein after therapy	Erythrocyturia before therapy	Erythrocyturia after therapy
1	19	M	[illegible]	1	10	0	1.0	84	138	920	713	0.0[illegible]	0.[illegible]	2.[illegible]	0.		
2	20	M		½	12	1	0.[illegible]	71	118	795	968	0.0[illegible]	0.[illegible]	[illegible]	0.[illegible]		–
3	21	M		190	5	4.[illegible]	6	22^{T}	12^{T}					5.[illegible]	6.[illegible]		
4	27	M		1	12	1	0	62	113	810	557	0.0[illegible]	0.[illegible]	4	0		
5	34	M		72	5	1	1.0	51	88	464	394	0.	0.[illegible]	0.[illegible]	0.[illegible]		
6	43	M		5	18	1	1	44	80	338	340	0.	0.[illegible]	0.[illegible]	0.[illegible]		
7	19	F		4	18	0.[illegible]	1	62^{T}	98^{T}					6	2.[illegible]		
8	20	F		120	21	0.[illegible]	0.	59	89	368	464	0.	0.20	0	0		
9	20	F		8	31	0	0.[illegible]	88	113	799	759	0	0.[illegible]	1	0		
10	28	F		168	38	1.[illegible]	1	49	44	317	273	0.[illegible]	0.[illegible]	2.	2.[illegible]	–	
11	30	F		120	3	0.[illegible]	0.7	132		778		0		6	5		
12	32	F		11	3	1	1.[illegible]	72	60	382	320	0.[illegible]	0.1	0.	0.5		
13	34	F		108	3	3.3	3	12	21	144	145	0.05	0.[illegible]	7	12		
14	36	F		8	22	1	0	39	116	549	689	0.05	0.[illegible]	10	0.[illegible]		
15	58	F		12	12	1	1.3		48		275		0	1.	0.[illegible]		
16	28	M	[illegible]	108	3	0.[illegible]	1	71	43	549	270	0.	0.[illegible]	8	13		
17	26	F		96	13	1.0	0	95	89	842	811	0	0.	4	1.[illegible]		
18	58	F	[illegible]	>10 yr	9	0	1.3	75	32	724	284	0.0	0.1	8	16		
19	26	M		5	6	0	0.	119	105	782	604	0	0.17	1	0.[illegible]		–
20	47	F		1	11	0	1	69^{T}	[illegible]		693		0.6	8	0		
21	20	F		5	4	0	1.0	110	103	932	884	0.[illegible]	0	4	3		
22	33	M	[illegible]	108	32	1	1	89	72	435	428	0.	0.	1	0.[illegible]		
23	56	M	[illegible]	7	8	2	2.[illegible]	21^{T}	25^{T}					3	0.[illegible]		
24	29	M	[illegible]	190	30	0.[illegible]	0	41	85	447	620	0.0[illegible]	0	5	4		
25	29	F		11	6	2	2	18	22	280	148	0.0[illegible]	0.[illegible]	7	7		

up time and to the fact that some of the patients were followed at other hospitals, we have not been able to examine the patients at equal follow-up intervals. This also explains the lack of clearance values in two patients.

Evaluation of the therapeutic effect was based upon the clinical state of the patient and upon changes of the above mentioned parameters. We have regarded change in inulin clearance of 10 ml/min or more as significant difference (6). Erythrocyturia has been determined either by the quantitative method of Addis or in the routine urinary sediment. Protein excretion was determined on 24-hour urinary specimens (13), and change in proteinuria of more than 50% has been regarded as significant. Most of the figures given are mean values of several observations on consecutive days.

RESULTS

A significant improvement in inulin clearance was observed in 11 patients, 6 of whom had a history of less than 6 months. Another . patients with a short history had clearance values within normal limits both before and after treatment. No significant change of subnormal clearance values was observed in 6 out of 10 patients with a history of more than 6 months, while a significant reduction of inulin clearance was found in the other 4 patients. Proteinuria was reduced in 11 patients, 6 of whom with a short history unchanged in 11 patients, . with a short history and increased in 3 patients, all with a long history. Twenty-two had erythrocyturia before treatment, and 9 after treatment.

Improvement in inulin clearance and/or proteinuria has been regarded as a positive result of the treatment and has been obtained in 12 patients, 8 of whom had a history of less than 6 months. A steady state has been maintained in 8 patients, while the condition in 5 patients has deteriorated.

The filtration fraction was initially low in all cases but one, and characteristically very low in patients with acute glomerulonephritis and lupus nephritis. The filtration fraction was wholly or partly normalized during treatment in 15 out of 19 with repeated determination of inulin and PAH clearances. With advanced renal impairment the figures of the filtration fraction are of less value, since the PAH clearance does not then constitute a reliable estimate of the renal plasma flow.

The therapeutical results in relation to the microscopic picture at biopsy (Table II) showed that 9 patients out of 15 with proliferative glomeru-

Table II. *Response to therapy related to duration of history*

Histopathological diagnosis	< 6 months			6 months 3 years			> 3 years		
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Proliferative glomerulonephritis	5			2	2		2	2	2
Membranous glomerulonephritis								1	2
Minimal change glomerulonephritis	2	1							
Lupus nephritis							1		1

lonephritis improved on therapy 4 maintained a steady state, while 2 demonstrated progress. Two of the patients who improved had histories of no less than 6 and 10 years respectively Two of three patients with membranous glomerulonephritis showed progression of the disease, while in one patient no change was found. Two of the patients with minimal change abnormalities improved, and the third maintained a status quo. One of the two patients with systemic lupus nephritis improved significantly while the other patient clinically ran a progressive course and eventually died. In one patient no biopsy was obtained and therapy did not change his condition. The two patients with repeat biopsy got a normalized renal function and morphologically reduced glomerular cellularity The two patients with reduced kidney size did not respond to therapy

Complications to the immunosuppressive therapy have been few Some patients have complained of diffuse abdominal discomfort, but therapy was withdrawn in only one patient for this reason. In some cases a temporary Cushing's syndrome developed but subsided when the steroid dosage was reduced. Likewise leucopenia has always been reversible, but it caused withdrawal of therapy in one case (no. 25). In case 12 the renal function decreased and this patient developed multiple abscesses in both kidneys during treatment. She had a history of mild urinary infection before the appearance of glomerulonephritis, and the immunosuppressive treatment was probably the main contributing factor to the development of the abscesses. Pregnancy was the cause of withdrawal in one patient.

DISCUSSION

Evaluation of the results of immunosuppressive therapy in glomerulonephritis is difficult. The length of history before therapy the natural course of the disease and the degree of irreversible tissue damage already present at the start of treatment are factors of importance. A maintained level of renal function in a case with a previous progressive downhill course could therefore be regarded as a favourable response. Thus the course in cases 10 and 12 has been considered as positive responses to the treatment. The positive results seem even more convincing when a long-term downhill course has changed into an improvement (cases 5 8, 14 and 24). On the other hand an improvement or even normalization of the clinical condition and renal function could sometimes be equally well ascribed to the natural course of the disease. In the present study this applies to cases 1 2, 4, 7 19 and 20 in whom the renal function at the start of treatment was within normal limits or in whom the duration of the disease before treatment was too short to predict the spontaneous development without therapy However in case 9 there was a history of 8 months with an insidious onset of symptoms and improvement after 31 months of treatment when this was withdrawn at the patient's wish. A follow-up investigation 9 months later showed a moderate but significant decrease in function and we intend to resume therapy The further course in this patient will be interesting. In other studies (9) it is even indicated that a slowly falling renal function may represent a beneficial response to immunosuppression if withdrawal of therapy causes abrupt deterioration and reinstitution of therapy reverses the course. It is desirable to start treatment before irreversible lesions have been established, but an early introduction of immunosuppressive therapy may unnecessarily anticipate a spontaneous remission.

The best results were found among patients

with either proliferative or minimal change glomerulonephritis, which is in accordance with earlier investigations (1 10). It is noticeable that four out of six patients with a history of more than 3 years could be judged as showing positive responses. Thus a long history does not seem to disqualify a therapeutic trial if the kidney size is not reduced. Two of our three patients with membranous glomerulonephritis failed to respond to therapy and the results in most other studies seem to be disappointing. A history of more than 3 years in our three cases may have been an additional unfavourable factor However scattered positive results of immunosuppressive therapy in membranous glomerulonephritis make it at present unreasonable to exclude such cases from clinical trials. As for lupus nephritis, there seems to be general agreement that corticosteroids alone or combined with cytotoxic agents, such as azathioprine, are often beneficial (5 8, 11)

How long should the treatment be pursued, if the patient is unresponsive to treatment, until it can be considered a definite failure? In the literature we have found no evidence that a prolonged course of treatment can be expected to give a positive response if no improvement at all is discernible after 3 or 4 months. In some unresponsive cases we have continued longer without effect, but we have seen no improvement starting after that period.

The other question which arises is how long should the treatment be continued after complete remission or an established steady state? There seems to be no convincing answer Immunofluorescent studies on repeat biopsies may be of great value for this decision. Some investigators have demonstrated that clinical improvement runs parallel to decreasing immunofluorescent deposits (10). However a decrease in glomerular deposition of IgG and complement is not invariably accompanied by clinical improvement (5). Other immunological studies have given similar results. Thus Swanson and Schwartz (14) demonstrated that there was no correlation between the extent of immunosuppression and clinical effectiveness of azathioprine and amethopterin. They investigated primary and secondary immune responses, delayed hypersensitivity immunoglobulin levels and isoagglutinin titres.

In the present study serum complement levels had been determined in 19 patients. Six out of eight patients with low complement levels showed a good therapeutic response, while the other two were unresponsive. Among patients with normal complement there were some with a positive response.

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CYTOGENETIC STUDIES IN ACUTE MYELOID LEUKAEMIA

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Abstract. Cytogenetic studies have been carried out on bone marrow aspirates from 50 patients with acute myeloid leukaemia (AML). Abnormal stem lines were present in 22 patients, the remainder had a diploid chromosome complement. In all patients the majority of the metaphases had a blurred appearance and in many of the bone marrow aspirates studied before therapy the blurred metaphases showed chromatid breaks. Several bone marrow aspirates were obtained from six patients with a diploid chromosome complement. In none of these was the emergence of an abnormal cell line seen. In seven patients abnormal stem lines were demonstrated before the diagnosis of AML could be made. Based on the present results and on a review of the relevant literature, some general cytogenetic features are emphasized which have contributed to a better understanding of the leukaemic process. Finally, the significance of cytogenetic studies in the diagnosis and therapeutic handling of leukaemia is discussed.

Although the significance of chromosome abnormalities in acute leukaemia remains obscure, cytogenetic studies have been able to elucidate several aspects of the disease. This report presents the cytogenetic findings in 50 patients with acute myeloid leukaemia (AML) and reviews the cytogenetic literature on AML in order to discuss both the relation of the chromosome abnormalities to the leukaemic process and their practical clinical significance.

MATERIAL AND METHODS

Between Sept. 1964 and Dec. 1970 bone marrow aspirates were studied from 50 patients with AML and its variants (acute erythroleukaemia and monocytic leukaemia). Their ages ranged from 8 to 75 years, 24 being males and 26 females. Cytogenetic findings in 22 of the patients have previously been reported (6). All the patients were in relapse when first studied. Forty-four of the patients were studied before therapy, the remainder had received therapy with various cytostatics. Repeated bone marrow cytogenetic studies were performed in 14 patients. The chromosome preparations were made according to the methods described by Tjio and Whang (19) and Lam-Po-Tang (12).

RESULTS

Three types of abnormalities of the chromosome complement were seen, viz. abnormal stem lines, blurring of the chromatin, and structural aberrations.

Abnormal stem lines. Twenty-two of the 50 patients had abnormal stem lines in the bone marrow. The remainder had a diploid chromosome complement. The abnormal clones were hypodiploid in nine and hyperdiploid in eight patients. Six patients had a pseudodiploid stem line (Table I). Chromosome studies were made more than once in eight patients with abnormal stem lines in the bone marrow. The abnormal complement was not replaced by a normal diploid complement in any of these patients—unless a complete remission was obtained. In one of the patients (no. 3) a hypodiploid stem line was present when the patient was first studied. In remission a normal diploid complement was found. In relapse a new pseudodiploid clone emerged—closely related to the original hypodiploid clone. Six of the patients with a diploid chromosome complement were studied more than once during the course of the disease. Three of the patients were reexamined 13, 17 and 4 months, respectively, after the first study. Development of an abnormal stem line did not take place in any case.

Blurring of the chromatin. It was a characteristic feature in all the patients that the chromatin

Table I. *Chromosomal findings in 22 cases of AML with abnormal stem lines*

Case no.	Age (y.)	Sex	Date	Cells counted	Chromosome number <41	41	42	43	44	45	46	46*	47	48	49	50	Abnormal metaphases (%)
1	45	♀	22.9.64	19							3	16					84
			27 10.64	36							3	33					92
2	75	♀	10.5.65	50	2	6	36	3	1	1	1						98
			9.6.65	50	4	5	37	4									100
			31 7.65	50	5	1	39	4	1								100
3	49	♀	3.6.65	50					4	38	8						84
			23.7.65	50						4	46						4
			29.9.65	19				1		2	14	2					11
			5.1.66	40						1	7	32					83
			24.3.66	39						3	2	34					87
4	50	♂	30.6 65	50	3	1		1	2	41	2						96
			9 11.65	50	1		2	1	5	40	1						98
5	75	♂	2.10.65	50					4	6	31		9				18
			9.11.65	50				3	1	17	24		5				[illegible]
6	70	♀	23.12.65	44	6	21	9	5	1		2						96
7	70	♂	23.12.65	19					1		2		16				84
			27 1 66	50					1		2		47				94
8	35	♂	21.2.66	50	1	2		3	42	2							100
			1 4.66	50	1	2	3	17	24	1	2						96
9	8	♂	7.6.66	29						2	10		16	1			59
10	73	♂	2.12.66	27					1	2		23	1				96
11	54	♀	6.10.67	24	3		1		12	8							100
12	44	♂	15.8.68	50							11	39					78
13	70	♂	31 10 68	50		1	1	7	40	1							100
14	65	♂	2.1.69	50							5		45				90
15	60	♀	23.5.69	50	1	1		1	3	1		34	1		1	7	100
16	68	♀	30.1.69	50		4		4	1	5	31	5					10
			6.3.69	50		2			2	3	32	11					22
17	69	♂	18.2.70	50	2				1		4		42	1			88
18	67	♂	15.5.70	50		2	1	2	7	34	3			1			94
19	62	♂	29.6.70	50	3		2			1	3		1	1	4	35	82
20	69	♀	14 7 70	50	2		2	3	2	35	1				1	4	96
21	23	♀	4.9 70	50		1	1		1	2	31		13	1			30
22	65	♂	12 10.70	50	1	1		1	2	7	21		17				36

* Pseudodiploid metaphases belonging to the abnormal stem line.

in most of the metaphases obtained in relapse had a blurred appearance with an ill-defined boundary of the chromatids (Fig. 1). In patients with abnormal stem lines it was characteristic that the aneuploid or pseudodiploid metaphases had blurred chromosomes, whereas many of the diploid metaphases, which are often also present in the same aspirates, had a normal chromatin appearance. In the patients who had not abnormal stem lines in the bone marrow both diploid metaphases with blurred chromatin and diploid metaphases with normal structure of the chromatin were present at the same time. In patients studied in remission, only metaphases with normal chromatin were seen (Fig. 2)· in relapse the chromosomes of most metaphases had again a blurred appearance.

Structural aberrations. Structural chromosome abnormalities were seen in 19 of the 44 patients studied before therapy with cytostatics. They were almost exclusively chromatid breaks, but a few breaks of the chromosome type were also present. The proportion of metaphases with structural aberrations in the bone marrow aspirates varied from 6 to 39 %. In ten control patients without a haematological disorder structural aberrations were found in 1 7 % of 350 metaphases studied. In none of these controls were there structural aberrations in more than 4 % of the metaphases. It is a characteristic feature of the patients of the present series that structural aberrations are almost exclusively seen in the aneuploid and diploid metaphases which have a blurred chromatin structure.

The cytogenetic study had a practical diagnostic

significance in seven patients who had abnormal stem lines in the bone marrow but in whom the diagnosis of AML could not be made on morphological criteria at admission.

The patients of the present series had been treated so differently that no attempt was made to find a possible correlation between the cytogenetic findings and course of the disease.

DISCUSSION

The results of more than ten years cytogenetic work in leukaemia are manifold and often contradictory. Nevertheless several general aspects have appeared, which may lead to a better understanding of the relation between the leukaemic process and the abnormal chromosome complement and its significance for the diagnosis and therapeutic handling of leukaemia. Some of these are summarized below.

1. Abnormal stem lines were present in 2_ of 50 patients of this series. Table II shows the results of some previous cytogenetic studies in AML and acute lymphocytic leukaemia (ALL). It is seen that between 40 and 50% of the patients from both categories have abnormal stem lines in the bone marrow. Table III shows that hypodiploid and hyperdiploid stem lines occur with approximately equal frequency in patients with AML, while pseudodiploid clones are rarely seen. Conversely in patients with ALL, abnormal stem lines are almost exclusively hyperdiploid—only four patients with hypodiploid clones have been described (4, 20).

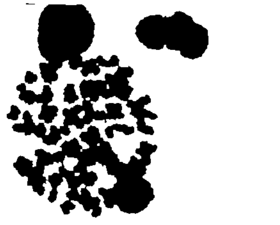


Fig. 1. Pseudodiploid metaphase with ill-defined and blurred appearance.

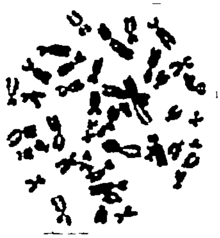


Fig. 2. Diploid metaphase with well-defined chromatids obtained from patient in remission.

2. The present report confirms earlier findings that, in patients with AML who have a normal diploid chromosome complement at the time of diagnosis, subsequent bone marrow studies do not ordinarily show the development of abnormal stem lines (6, 8). The emergence of abnormal clones during the course of the disease has been described only in two patients with AML (2, 18). However in these cases the possibility cannot be excluded that the reason for finding a normal chromosome complement originally is that only non-leukaemic bone marrow metaphases were studied. Recently however Gunz et al. (3) have convincingly demonstrated the emergence of aneuploid cell lines in a patient with acute undifferentiated leukaemia. At the same time it has been demonstrated that, if an abnormal stem line is established in a patient with AML, this clone will not be replaced by normal diploid cells at a later stage of the disease—unless there is a remission. Evolution of a new clone in an already cytogenetically abnormal bone marrow—as in case 3 of the present series—has been described in a few patients with AML (1, 11).

In remission metaphases belonging to the abnormal stem line cannot be demonstrated in the bone marrow: at this time the marrow shows a normal diploid chromosome complement. In renewed relapse the same abnormal stem line as was present before remission reappears in the bone

Table II. *The frequency of abnormal stem lines in bone marrow aspirates from 383 patients with AML and ALL*

Source of data	AML No. of cases examined	AML No. of cases with abnormal stem lines	ALL No. of cases examined	ALL No. of cases with abnormal stem lines
Sandberg (16)	102	50	102	54
Whang-Peng et al. (20)	—	—	26	13
Whang-Peng et al. (21)	103	30	—	—
Present series	50	22	—	—
Total	255	102	128	67

marrow (14 15). In case 3 of the present report the emergence of a new abnormal stem line—related to the original abnormal clone—took place.

3 Little attention has been paid to the fact that most metaphases from leukaemic bone marrow aspirates have a blurred and ill-defined appearance of the chromatids, which may often show breakage (6, 8 16, 18). The reason for the blurring of the chromosomes is unknown it may be related to an abnormal DNA-structure or abnormalities of the protein surrounding the chromatids. The results of the present study suggest that the metaphases with blurred chromosomes represent the leukaemic cell population, while the few metaphases with normal appearance of chromatin, which are most often present at the same time may indicate a non-leukaemic cell population which resides in the bone marrow together with the neoplastic clone.

4 Evidence has been presented that in AML the chromosome abnormalities are not confined to the myeloblasts but are also present in the erythroid precursors (9 10) Conversely the lymphocytes in the peripheral blood which are triggered into division by phytohaemagglutinin have a normal chromosome constitution (9).

5 Abnormal stem lines are often seen in the bone marrow aspirate at an early stage of the disease when the diagnosis of AML cannot yet be made on morphological criteria (6, 8, 18).

The above-mentioned data constitute most of our present knowledge of chromosomes and leukaemia. They suggest that in AML the bone marrow metaphases with blurred chromosomes constitute the leukaemic cell population, while the metaphases with normal chromatin represent the remnants of a non-leukaemic population, which exists in the bone marrow together with the neoplastic clone. In patients with abnormal stem lines in the bone marrow these probably correspond to the leukaemic cells, while the diploid metaphases which are most often present in an insignificant number may indicate the non-leukaemic clone. However in patients with a minor aneuploid cell line some of the diploid cells are likely to represent leukaemic cells which may remain diploid during the course of the disease.

The demonstration of the same abnormal chromosome complement in the myeloblasts and in the erythroblasts of patients with AML means that, at least in part, erythropoiesis in AML is not normal but directly affected by the leukaemic process. Thus AML is not a disease of the granulocytic cell line but rather a disorder of haemopoiesis in general (9 10). The abnormal chromosome complement has probably arisen in the common haemopoietic stem cell.

The cytogenetic data also indicate that the abnormal stem lines in AML are present at least at a very early stage of the disease and do not arise terminally However it has not yet been possible to solve the problem whether the chromosome abnormalities are causally related to the leukaemia or whether they result from the neoplastic change.

Table III. *The ploidy of abnormal stem lines in 228 patients with AML and ALL*

Stem lines	AML (n)	ALL (n)
Hypodiploid	44	4
Pseudodiploid	11	2
Hyperdiploid	64	103

Little attention has been paid to the application of cytogenetic studies in the diagnosis and therapy of acute leukaemia. They may turn out to be a valuable diagnostic procedure in patients who present with anaemia and abnormal thrombocyte and/or leucocyte counts, and in whom the diagnosis of AML cannot be made from the morphology of the peripheral blood cells and bone marrow. In these patients the presence of an abnormal stem line in the bone marrow suggests a leukaemic state. Conversely the finding of a diploid chromosome constitution does not exclude a neoplastic process, as about 50 per cent of patients with AML have diploid chromosome complements. The demonstration of metaphases with blurred chromatin and chromatid breaks cannot yet be relied upon diagnostically because it is often difficult to evaluate whether the blurring of the chromatin is due to a leukaemic process or poor technique. The diagnostic significance of chromosome studies in AML may be increased considerably when two problems have been elucidated in the future. For one thing it must be clarified whether the presence of an abnormal stem line in the bone marrow is synonymous with a neoplastic process. This may be achieved by studying patients with potentially leukaemic disorders, viz. polycythaemia vera, myelofibrosis, aplastic anaemia, pancytopenia, and refractory anaemia, who may have cytogenetically abnormal clones in the bone marrow. Preliminary results indicate that abnormal stem lines in these disorders are associated with a high risk of developing frank AML (7, 13). Secondly an attempt must be made to arrive at a more certain evaluation of the blurring of the chromatin in leukaemic metaphases—possibly by means of electron-microscopic studies.

In the future, cytogenetic studies may turn out to be of value in the therapeutic handling of leukaemia. Thus repeated bone marrow studies may indicate whether a remission is complete or partial or whether relapse is imminent. However this must wait for automatized chromosome analysis, as a large number of metaphases must be studied in each aspirate.

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ANEMIA IN UREMIA

Hemolysis Evaluated by Common Methods and Application of the Bilirubin Turnover Method

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Abstract. One hundred and thirty-eight patients with renal insufficiency of varying degree have been studied with respect to anemia and signs of hemolysis. A control material is included in the study. 1) The patient group displayed normochromic anemia with only slightly subnormal mean corpuscular hemoglobin concentration. 2) A moderately elevated reticulocyte count together with a tendency to elevated endogenous carbon monoxide production indicated a certain degree of hemolysis in the uremic patient group compared with the controls. Patients with chronic pyelonephritis and active urinary infection displayed an elevated reticulocyte count ($p < 0.001$) and lower haptoglobin values ($0.05 > p > 0.01$) than non-infected uremic patients. Thus we consider these infections to be a hemolysis-promoting factor in patients with renal insufficiency. 3) In men there was a positive correlation between the concentrations of serum creatinine versus serum iron concentration and the saturation percentage of the total iron binding capacity. In the light of cited iron kinetic data, we consider this correlation an indicator of depressed erythropoiesis. 4) A selected group of severely ill uremic patients were studied with respect to their erythrocyte life span by the bilirubin turnover method. As a group, they displayed a shortened erythrocyte life span in comparison with an earlier published control group.

The anemia which is found in patients with renal failure has been considered to be due mainly to three factors: toxic depression of the erythropoiesis (6, 10, 15–22, 24–35), insufficient production of erythropoietin (25), and hemolysis (6, 15, 17–22, 24, 25, 27–28). Some of the factors known to promote hemolysis in these patients are

1. acidosis, considered to influence the glucose metabolism of the erythrocyte, with resulting early ageing of the red cell (23)
2. infections, with a rather complex mechanism (3)
3. drug action (5),
4. microangiopathic hemolytic anemia (2).

Patients with the same degree of uremia as judged from the laboratory values often display a most varying degree of anemia and consequently a varying need for anti-anemic therapy. When blood transfusions have been given to patients with advanced renal failure, the hemoglobin concentration often rapidly returns to the pretransfusional level. This is a difficult problem, especially in connection with hemodialysis (10). In this study we have tried to correlate hematological values with the renal function in patients with uremia of varying degree in order to elucidate the influence of various factors of renal impairment, such as infection and degree of uremia, on the anemia. In a number of patients the erythrocyte survival time was studied by the bilirubin turnover method. The results suggest a positive influence on the erythrocyte destruction from active urinary tract infections; in addition, depressed erythropoiesis seems to be the most important anemia-promoting factor.

MATERIAL

Uremic patients, 77 men and 61 women, admitted to the Medical Department, Karolinska sjukhuset, 1964–1969, were chosen for correlative studies concerning hematological data and renal function. All patients had a serum creatinine concentration more than 2 mg/100 ml. They were investigated on admission, before hospital therapy had started. All were in-patients. The diagnoses are recorded in Table I and were confirmed by later autopsy in 40 cases and by needle-biopsy in another 8 cases. Patients with unspecified diagnoses were mostly those in

Table I. *Diagnoses of the patients before treatment*

	Men	Women
Chronic pyelonephritis	16	32
Papillitis necroticans	4	17
Glomerulonephritis	22	1
Nephrosclerosis	8	2
Polycystic kidneys	6	2
Diabetic nephropathy	1	2
Amyloidosis	2	0
SLE	1	0
Nephronophthisis	2	0
Renal tuberculosis	1	0
Oxalosis	1	0
Gouty nephropathy	1	0
Tubular necrosis	0	1
Postrenal uremia	1	1
Unspecified	11	3
	77	61

chronic nephritis in the case records and patho-anatomical separation into glomerulonephritis and pyelonephritis was not always possible. The age distribution of the patients is shown in Fig. 1 the degree of uremia on admission in Fig. 2. The hematological variables studied are: hemoglobin concentration, MCHC, reticulocyte count, serum iron concentration, total iron binding capacity haptoglobin concentration, and endogenous carbon monoxide production.

Hematological values from healthy controls are taken from an earlier publication (31), hemoglobin concentration was determined also in a group of healthy conscripts, 18 years of age.

METHODS

Hemoglobin concentration was determined in capillary blood as cyanmethemoglobin (16).

The volume of packed red cells was determined with an International hematocrit centrifuge, type M.B.

Reticulocytes were counted by a supravital staining method (20). One thousand cells were counted.

Serum iron concentration. The bathophenanthroline method (36), adapted for use with the autoanalyzer.

Total iron binding capacity. The serum was saturated with iron. The iron concentration was determined as described above.

Haptoglobin analyses were made by method described by Tarukoski (34).

Endogenous COHb production was determined at the Department of Clinical Physiology using the alveolar carbon monoxide method of Sjöstrand (29). Double determinations were made, and only values from non-smokers were registered. The control group concerning COHb analyses refers to the laboratory's own controls.

Serum creatinine concentration (12) was determined on the autoanalyzer.

Erythrocyte survival time from bilirubin turnover was determined by the method of Engstedt et al. (8).

Statistical methods. The statistical analysis was done by

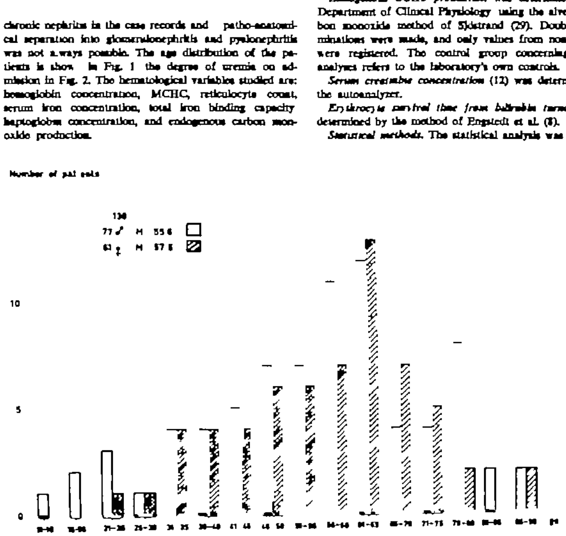


Fig. 1. Age distribution of the patients with renal insufficiency

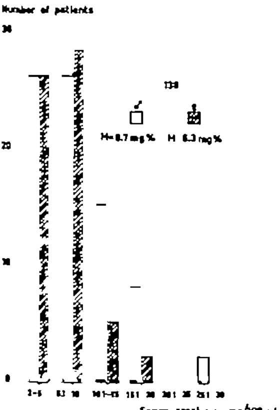


Fig. 2 Distribution of serum creatinine concentrations in the uremic patients.

generally accepted methods (30). The following significance levels are used: $0.05 > p > 0.01$ probably significant, ** $0.01 > p > 0.001$ significant, *** $p < 0.001$ highly significant.

RESULTS

Hematological values and signs of hemolysis

As shown in Table II the uremic patients had a subnormal mean hemoglobin concentration, 9.2 g/100 ml. The mean of the MCHC did not differ significantly when the alues for uremics and controls were compared. The uremics displayed elevated reticulocyte count, significant for the female group. The serum iron concentration was subnormal in the patient group, but there was a statistically significant difference only in the female groups. In both sexes the means of the TIBC concentration were at the lower limit for normal values and statistically different from the controls. The saturation of TIBC did not differ from a control group as regards the male uremic patients, but did differ in a highly significant way among the women. As will be discussed later the different degree of uremia when comparing the sexes may here be of some importance

The concentration of COHb was slightly elevated in the uremic patients and the difference was probably significant for the women, indicating a certain hemolysis.

A low haptoglobin concentration indicates he

Table II. *Hematological data. Means for uremic patients (u) and controls (c)*
Difference between means

		Males				Females			
			Mean	S D.	Difference		Mean	S D.	Difference
Hemoglobin		77	9.2	1 71		61	9 2	2.30	
(g/100 ml)		111	15.4	0.69	6.2*	20	12.2	0.6	3 0
MCHC	u	59	32.6	3 4		33	31 4	3.6	
(%)		14	33 1	0.83	0 5	21	32.1	0.9	0.7
Reticulocytes		52	2.1	1.54		32	2.5	2.0	
(%)		19	1.2	0.68	0.9	19	0.8	0.5	1 7
Serum iron		62	74	41		41	61	28	
(µg/100 ml)		11	98	51	24	19	102	40	41
TIBC		49	268	42		26	287	61	
(µg/100 ml)		11	352	89	84	14	330	43	43
Saturation of	u	49	28.6	16 0		26	21 6	8.7	
TIBC (%)		11	28.8	15.6	0.2	14	35.7	11.3	14.1
COHb		21	0 88	0.39		8	0.86	0.26	
(%)		91	0.75	0.20	0.13	34	0 63	0.11	0.23

*$0.05 > p > 0.01$ $0.01 > p > 0.001$. $p < 0.001$

Table III *Serum creatinine concentration correlated to various hematological variables in patients with uremia*

Variable	Sex	No. of observations	Correlation coefficient
Hemoglobin concentration	♂	76	−0.35
	♀	59	−0.35
Mean corpuscular hemoglobin concentration	♂	59	−0.09
	♀	33	−0.11
Reticulocyte count	♂	51	0.02
	♀	32	−0.04
Serum iron concentration	♂	61	0.49
	♀	41	0.01
Total iron binding capacity	♂	47	0.29
	♀	26	0.01
Saturation % of TIBC	♂	46	0.47
	♀	26	0.12
Endogenous COHb production	♀	24	−0.06
	♀	8	−0.09
Haptoglobin concentration	♂	38	−0.01
	♀	16	−0.08

$0.05 > p > 0.01$ $0.01 > p > 0.001$ $p < 0.001$

molysis at the time of drawing the blood sample (13). Patients from the present material with signs of actual hemolysis, with haptoglobin values ≤40 mg Hb/100 ml ($n = 16$) were compared with 36 patients with haptoglobin concentrations >40 mg Hb/100 ml. The patients with signs of actual hemolysis displayed a lower mean hemoglobin concentration (7.8 g/100 ml, S.D 1.9) compared with non-hemolytic patients (mean 9.1 S.D 1.9) They also had higher serum creatinine (10.6 mg/100 ml, S.D 6.1) than the non-hemolytic patients (8.7 S.D 6.2). The mean serum iron and iron saturation of the TIBC were the same for both groups. There was also a higher frequency of active urinary infections in the low-haptoglobin group: 13/16 compared with 13/36 in the group of patients with haptoglobin >40 mg Hb/100 m.

The influence of uremia on hematological values

We further examined how these hematological parameters correlated with uremia expressed as serum creatinine concentration (Table III and Figs. 3 4 5 6). The concentrations of hemoglobin and serum creatinine were negatively correlated, $r = -0.35$ and the serum iron concentration was positively correlated to the serum creatinine concentration among the uremic men. Serum iron was higher for those with high serum creatinine, $r = 0.49$ (Fig. 5) and the saturation of TIBC behaved in the same way $r = 0.47$ (Fig. 6). The same correlation was not valid for the women, but when the sexes were

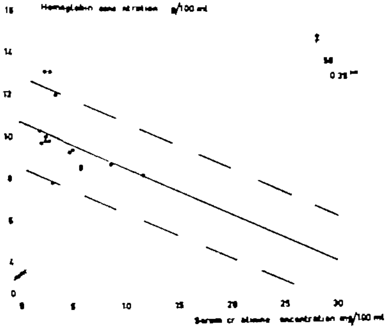


Fig. 3 Correlation between serum creatinine and hemoglobin concentrations in 59 uremic females.

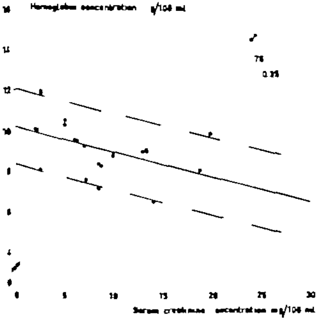


Fig. 4. Correlation between serum creatinine and hemoglobin concentrations in 76 uremic males.

lumped together there was still a good correlation for these parameters, for serum iron concentration $r = 0.40$ and for saturation of TIBC, $r = 0.46$ There was a slight positive correlation between serum creatinine concentration and TIBC in the male uremic group.

MCHC, reticulocyte count, haptoglobin and COHb were not influenced by serum creatinine concentration. There was no correlation between reticulocyte count and hemoglobin concentration, nor was there a statistical relation between serum iron and hemoglobin concentrations. When the patients with serum creatinine < 10 mg/100 ml were excluded, there was a very close negative correlation between hemoglobin and serum iron concentrations, $= -0.49$

Influence of infection on hemolysis

Patients with chronic pyelonephritis and signs of active urinary infection with positive urinary cultures were compared to non-pyelonephritic patients, with respect to their hematological values (Table IV). The pyelonephritic group had a higher mean reticulocyte count ($p < 0.001$) and lower mean haptoglobin concentration ($0.05 > p > 0.01$), indicating a possible hemolytic mecha-

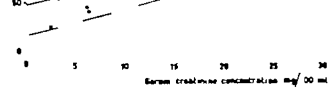


Fig. 5. Correlation between serum creatinine and iron concentrations in 61 uremic males.

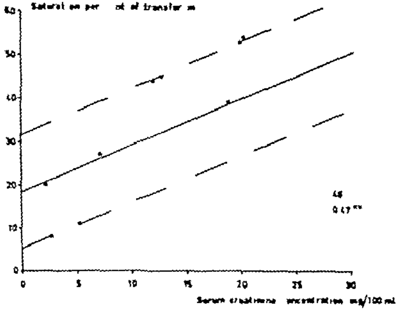


Fig. 6 Correlation between serum creatinine and saturation percentage of transferrin in 46 uremic males.

Table IV *Hematological data. Means for uremic patients with active urinary infection and pyelonephritis and for uremic patients with other diagnoses*

		Mean	S.D	Difference
Serum creatinine concentration (mg/100 ml)				
Pyelonephritis	48	6.81	4.4	
Other diagnoses	56	9.21	6.1	2.40
Hb (g/100 ml)				
Pyelonephritis	46	9.19	2.19	
Other diagnoses	56	9.28	2.16	0.09
MCHC (%)				
Pyelonephritis	26	32.3	2.7	
Other diagnoses	44	32.1	2.6	0 2
Reticulocytes (%)				
Pyelonephritis	29	3.5	2 4	
Other diagnoses	41	1 7	1.2	1 8
Serum iron (µg/100 ml)				
Pyelonephritis	34	76.1	42.7	
Other diagnoses	44	81 4	41 4	5.3
TIBC (µg/100 ml)				
Pyelonephritis	24	294.6	56.5	
Other diagnoses	36	265.9	39.0	28 7
Saturation of TIBC ( )				
Pyelonephritis	24	25.9	12.0	
Other diagnoses	37	31.8	15.8	5.9
COHb (%)				
Pyelonephritis	8	0.944	0.352	
Other diagnoses	19	0.903	0.398	0.041
Haptoglobin (mg Hb/100 ml)				
Pyelonephritis	16	80.4	76.7	
Other diagnoses	33	148.3	108.9	67.9

$0.05 > p > 0.01$ $p < 0.001$

nism operating in the infected group The difference of COHb production in the groups was not significant. The mean hemoglobin concentration, however was the same in both groups. When the patients treated with sulfa or nitrofurantoin were compared to those without such therapy there was no statistical difference between these hematological variables. In the same way the patients were studied regarding hypertension those with retinal changes of degree III–IV (20) did not differ from those without any traces of malignant hypertension. The acidotic patients with a CO_2-combining power less than 16 mEq/l also did not deviate from the hematological pattern of the less acidotic patients.

Erythrocyte survival study

Twelve patients witht advanced uremia were studied with special attention to the erythrocyte life span The method of bilirubin turnover (8) was used. The patients and the results of this part of the study are shown in Table V compared with results from healthy controls taken from the original publication (8) the erythrocyte life span *of the total uremic group is subnormal at the 1% level.* Conceivably the erythrocyte life span is positively related to the hemoglobin ($r = 0.54$) and packed cell volume ($= 0.52$*). *There is no* correlation between the degree of uremia and erythrocyte life span ($r = -0.32$) in accordance with the large material (221 cases) collected by

Table V *Diagnostic and hematological data for twelve patients, studied with respect to erythrocyte life span with bilirubin turnover*

Pat. no.	Sex	Age (y)	Diagnoses	Hb conc (g/100 ml)	Packed cell vol. (%)	MCHC (%)	Serum creatinine (mg/100 ml)	Erythrocyte life span (d)
1	♀	73	Amyloidosis, sec. to myelomatosis	8.1	24	34	7.5	33
2	♂	48	Chronic glomerulonephritis	10.3	31	32	5.0	76
3	♂	59	Phenacetin kidney disease	10.2	32	32	7.5	34
3a	♂	60	Phenacetin kidney disease	7.4	34	31	11.2	59
4	♂	72	Chronic glomerulonephritis	7.0	24	29	7.9	55
5	♀	57	Polycystic kidneys	8.6	28	31	5.2	72
5	♀	58	Polycystic kidneys	8.5	27	31	10.7	50
6	♂	53	Chronic glomerulonephritis	7.1	24	30	11.5	62
7	♂	32	Nephrosclerosis	14.5	39	37	6.1	149
8	♂	23	Polycystic kidneys, Mb Pringle	10.4	32	33	11.0	53
9	♀	34	Chronic pyelonephritis	10.3	32	32	8.2	120
10	♂	15	Juvenile nephronophthisis	10.7	31	35	11.0	56
11	♂	18	Oxalosis	7.4	23	32	10.1	76
11	♂	18	Oxalosis	8.5	25	34	13.3	69
12	♂	25	Juvenile nephronophthisis	11.7	34	34	6.0	72

Erslev (9) from the literature and his own figures. Furthermore the reticulocyte count, serum iron, TIBC, saturation of TIBC, haptoglobin and endogenous carbon monoxide production give no certain correlation to the erythrocyte life span figures.

DISCUSSION

Renal anemia is normochromic and normocytic, as pointed out by many authors. The mean corpuscular hemoglobin concentration in the present series of patients displayed a larger deviation than in the controls, but the mean value was within the normal limits. From Fig. 1 it is clear that about two-thirds of the women were above fertile age. For that reason the signs of iron deficiency cannot entirely be ascribed to menstrual bleedings in the female uremic patients. There was no significant difference between the MCHC means when female and male uremics and controls were compared, but the uremic patients had slightly lower means (Table II). The mean serum iron concentration was lower in uremic females and males when compared with their controls. The mean total iron binding capacity was lower in the uremic groups compared with the controls, this would be an argument against an uncomplicated iron deficiency. The saturation percentage of the TIBC was identical in uremic and healthy men, but definitely subnormal in the uremic women, which would suggest that these women had a slight iron deficiency. However the mean value does not indicate an iron deficiency of higher degree as the iron deficiency saturation percentage is > 10% (1).

The decreased concentration of transferrin in the uremic groups makes the evaluation of iron deficiency rather difficult. Laurell (21) showed that the TIBC is subnormal in chronic inflammatory diseases, and of these uremic patients at least 50% had signs of infection (Table IV). A loss of β-1-globulin from the gastrointestinal tract has been shown to exist by in vivo labelling of β-1-globulin with $^{51}Cr\ Cl_3$ in patients with renal insufficiency (14). Whether this intestinal loss is responsible for the subnormal serum concentration of transferrin is not clear. The decrease of the TIBC in the uremic patients might partly be due to chronic inflammation (pyelonephritis) partly to the intestinal loss referred to. In the present material there is a small percentage of patients with nephrotic syndrome, with renal losses of transferrin, and 63 of the 138 patients had signs of urinary infection on admission. Thus several factors may contribute to decreased TIBC figures in patients with renal insufficiency.

Hemolysis

The present patient material displays as a whole no impressive signs of hemolysis. However when the patients are separated into pyelonephritic

patients with active urinary tract infections and patients with other diagnoses (Table IV) the results indicate that hemolysis exists in patients with infection. The elevated reticulocyte counts in patients with active pyelonephritis and the dif ference in haptoglobin concentration in infected and non-infected uremic patients (Table IV) may be taken as signs of increased hemolysis in the infected group.

When the usefulness of the hemolysis parameters is considered, certain difficulties arise. The reticulocyte count may be elevated in iron deficiency anemia during therapy In patients with chronic inflammatory diseases, i.e. rheumatoid arthritis, elevated reticulocyte counts have been registered (31) Thus the counting of reticulocytes alone is not sufficient for indicating hemolysis, other hemolysis variables must be con sidered simultaneously Haptoglobin is not always a very useful laboratory test in chronic infections, as the α_2-globulin is elevated as a rule in inflammations, thus giving an unspecific rise of haptoglobin (13 26)

The endogenous production of carbon mon oxide is a very sensitive indicator of destruction of eyrthrocytes (7 29). Our limited number of COHb observations is due to certain limitations in the application of the method: too high values in smoking patients and difficulties for very disabled persons to accept and perform the ex amination The bilirubin turnover method for estimating the erythrocyte life span has been used in a selected series of patients and proved to be valuable in clinical routine work (8) It has the advantage of being rapid, the calculations can be made from determinations of blood samples taken during one hour the test can be repeated after a short time (hours).

We have not been able to show that sulfa therapy of urinary tract infections generally induces hemolysis. However in four of our patients observed in the clinic, heavy hemolysis was seen in connection with sulfa therapy the hemolysis subsiding when the sulfa preparation was withdrawn.

Correlation studies

There were significant correlations in men between the serum creatinine concentration, on the one hand, and the serum iron concentration and saturation percentage of the TIBC on the other This correlation was not significant when the female group was studied. The same difference between the sexes, and in the same direction, was found in patients with rheumatoid arthritis when serum iron was correlated to variables assessing clinical activity of the rheumatic disease (32) The present data cannot give an explanation of this sex difference. Possibly the existence of an iron deficiency anemia in women might influence the difference. During their fertile age women display a larger tendency to live with empty iron stores, compared with men, and bad nutrition during the development of uremia certainly does not improve the iron stores of female patients.

Several authors (4 15 22, 27 28) have regarded hemolysis as an important factor in renal anemia during the more advanced stages of renal failure. Desforges et al. (6) on the other hand, found no such relation between hemolysis and stage of uremia. There was no evidence in our material of an increased hemolysis in higher degree of uremia. Factors such as acidosis, urinary infection, treatment with sulfa preparations, hypertension, had no effect on the hemolysis when all the patients as a group were compared with controls. Separation of the patient group into the sub-groups of infected pyelonephritic versus non-infected patients revealed increased hemolysis in the infected patients. Single cases with sulfa-induced severe hemolysis were observed in the present material.

An interesting fact was the rise in serum iron concentration and saturation percentage of the TIBC with increasing serum creatinine concentration. Eschbach et al. (11) demonstrated iron over load in some patients with hemosiderosis in the liver and increased saturation percentage of the TIBC. This iron overload could be reduced after dialysis as a result of improved erythropoiesis. According to Kaye (17) and Kurtides et al. (19) the serum iron concentration tends to decrease as the renal failure advances. Loge et al. (22) found no consistent pattern of iron in uremic patients. Our positive correlation between the creatinine concentration and serum iron and saturation of TIBC is in concordance with the ferrokinetic studies of for instance, Kurtides et al. (19), Kaye (17) and Eschbach et al. (10). As the uremia advances, the decreased erythropoiesis (depressed erythropoiesis) results in a larger circulating iron

pool, which is not utilized for the production of erythrocytes, in spite of an advanced anemia.

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HYPERPARATHYROIDISM AND MAXIMAL GLUCOSE REABSORPTION

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Abstract. The maximal tubular reabsorptive capacity for glucose (TmG) has been determined in patients admitted to the hospital for suspected hyperparathyroidism. One group consisted of 19 cases with proved hyperparathyroidism. The other group consisted of 14 cases with normal serum calcium concentration on repeated determinations, and in four of these patients normal parathyroid glands were found at neck exploration. No significant difference in TmG was found between the groups, either when comparing the absolute values of TmG or when comparing TmG at equal values of glomerular filtration rate (GFR). Thus the results do not support the previously stated opinion that determination of the maximal tubular reabsorptive capacity for glucose is of value as a diagnostic aid in hyperparathyroidism.

Determination of the maximal tubular reabsorptive capacity for glucose (TmG) is a rather complicated procedure and is not frequently used in clinical work. However, recent observations indicate that a measurement of the renal threshold for glucose, as determined by the ratio between TmG and the glomerular filtration rate (GFR), might be of diagnostic value in parathyroid disease (5, 6, 7). The aim of the present investigation has been to test the validity of this suggestion for the diagnosis of hyperparathyroidism.

MATERIAL

The material consists of patients examined for suspected primary hyperparathyroidism in the years 1967–1969 at Sahlgren's Hospital, Göteborg, Sweden. The material comprises two groups.

Group A consists of 19 cases operated upon for hyperparathyroidism, 8 men (average age 41 years) and 11 women (average age 50 years). The diagnosis of hyperparathyroidism was based upon an increased serum calcium concentration. The mean calcium levels (mean of 8 to 10 determinations) in these patients ranged from 5.1–6.3 mEq/l. Eleven cases had renal calculi. No changes in the skeletal system associated with hyperparathyroidism were seen radiologically in any of these cases. At operation 17 patients showed a single adenoma of the parathyroids, one case mixed adenoma and hyperplasia, and one case primary chief cell hyperplasia. After operation all patients became normocalcaemic.

Group B consists of 14 patients. Clinical data are presented in Table I.

In four cases the parathyroids were explored and four normal parathyroid glands were found, confirmed by histology of at least one parathyroid gland from each case. These four patients had renal calculi but no changes in the skeletal system on X-ray. One of them was examined twice (pre- and postoperatively). Another patient, who is included in group A with regard to preoperative examination, belongs to group B after removal of a parathyroid adenoma.

Nine patients were not subjected to exploration of the parathyroids. With the exception of two patients, all had a history of renal stones. Two patients were found to have medullary sponge kidney which may account for the history of renal stones. Three patients did not undergo radiological examination of the skeletal system; the rest showed no radiological changes in the skeleton associated with hyperparathyroidism. One patient had Paget's disease. Three patients had a few slightly elevated serum calcium values, but on later occasions repeated determinations of the serum calcium were within the normal range.

METHODS

Procedure

The studies were performed in the morning after the subjects had had a light breakfast consisting of 1–2 sandwiches and 500 ml of weak tea. Each study included 7 clearance periods. During the first 3 periods, each of 15 min duration, simultaneous determinations of inulin, ^{51}Cr-EDTA and phosphate clearances were performed, and during the last 4 periods determinations of ^{51}Cr-EDTA clearance and TmG. After a priming dose of inulin (50 mg/kg) and ^{51}Cr-EDTA (50 μc) continuous infusion of these compounds was started by means of a constant infusion pump. The sustaining dose of ^{51}Cr-EDTA was 0.4 μc/min. The infusion rate of inulin was chosen to give a plasma concentration of about 25 mg/100 ml. The equilibration period was at least 30 min. Before the last 4

Table I. *Serum calcium levels and diagnoses in the patients assumed to have a normal parathyroid function (group B)*

NC = normocalcaemia

Age (y)	Sex	Serum Ca (mEq/l) Mean	Range	No. of obs.	Diagnoses, duration of symptoms (y)	Follow-up
Normal parathyroid glands found at exploration						
38	♂	4.9	4.6–5.4	18	Recurrent renal calculi (7)	No symptoms NC
35	♂	4.9	4.6–5.5	24	Recurrent renal calculi (7)[a]	Renal colic NC
49	♂	5.0	4.8–5.1	12	Recurrent renal calculi (20)	No symptoms NC
44	♂	5.0	4.6–5.8	20	Renal calculi (0.5), unilateral thyroid plasia	No symptoms NC
Normal parathyroid glands left after removal of an adenoma						
48	♀	4.7	—	1	Control 4 months after operation	No symptoms NC
Not explored, parathyroid function assumed to be normal						
46	♂	4.8	4.5–5.0	8	Osteoporosis, spontaneous fractures (13)	New fractures NC
43	♂	4.7	4.5–5.0	9	Recurrent renal calculi (23), gastritis, hypertension	No symptoms
37	♂	4.9	4.8–5.0	7	Recurrent renal calculi (1), medullary sponge kidney	No symptoms
52	♀	5.1	4.9–5.4	14	Recurrent renal calculi (24), peptic ulcer (9)	No symptoms NC
35	♂	4.9	4.7–5.0	6	Hypertension (2)	Not examined
56	♂	4.9	4.7–5.1	5	Peptic ulcer (35), recurrent renal calculi (28)	Renal colic
51	♂	4.9	4.4–5.5	10	Renal calculi (0.5), medullary sponge kidneys	No symptoms NC
56	♂	4.9	4.7–5.3	11	Recurrent renal calculi (25), peptic ulcer (4), Paget disease	Renal colic NC
30	♂	4.6	4.4–4.9	20	Recurrent renal calculi (11), gastritis	No symptoms

[a] Examined twice (pre- and postoperatively).

clearance periods a priming dose of glucose (0.5 g/kg b.w.t.) was given. The sustaining dose was 1.25 g/min, given as solution containing 30 g glucose/100 ml. The equilibration time was 20 min and the duration of each clearance period 10 min.

The infusions were performed through a catheter introduced into an antecubital vein, and blood sampling through another catheter in a vein in the opposite arm. An intraarterial catheter was introduced into a brachial artery in 14 of the subjects for determination of the arterial glucose concentration. Blood sampling was performed in the middle of each clearance period.

The urine was collected through a bladder catheter. At the end of each period the bladder was rinsed with 100 ml sterile water and thoroughly emptied by means of air insufflation and gentle suprapubic pressure.

Data handling

TmG was calculated according to the equation: TmG (mg/min) = PG (mg/ml) · GFR (ml/min) − UG (mg/ml) · V (ml/min), where PG = plasma glucose concentration, GFR = glomerular filtration rate, UG = urine glucose concentration, V = urine flow. During the measurement of TmG the glomerular filtration rate was estimated from the clearance of ^{51}Cr-EDTA. This value could be converted to the inulin clearance value since a close correlation was established between these parameters (Fig. 1). The results presented in Fig. 1 are based on 143 simultaneously measured clearance values for inulin and ^{51}Cr EDTA, of which 102 were recorded in the present study.

In 14 of the subjects (56 observations) the arterial and venous glucose concentrations were measured simultaneously. The correlation between these parameters is given in Fig. 2. Using this equation the venous glucose concentration was converted to arterial glucose concentration, which was used in the calculation of the tubular load.

Fig. 3 demonstrates that there were no significant differences between the two groups in either of the 4 clearance periods when the tubular reabsorptive capacity was determined.

Analytical methods

Inulin was determined according to Hubbard and Loomis (8). ^{51}Cr EDTA was counted in an automatic well-type scintillation counter (Selektronik, Sweden). Glucose in blood and urine was measured by a glucose-oxidase method after precipitation of the proteins with perchloric acid (9). Serum calcium was determined with an Eppendorf flame photometer. The standardization was continuously checked with commercial test sera. The normal range ($M \pm 2$ S.D.) with this method is 4.3–5.1 mEq/l. Serum and urinary phosphate were determined by the Fiske and SubbaRow procedure (3) adapted for the AutoAnalyzer (2).

Statistics

Student *t*-test was employed for testing differences between means. Simple and multiple regression analyses were performed according to the principles given by Hald (4), and differences between regressions according to Angervall et al. (1).

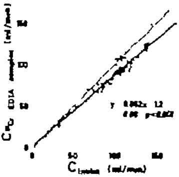


Fig. 1. Correlation between inulin and ^{51}Cr-EDTA-complex clearance determined simultaneously in 143 separate clearance periods.

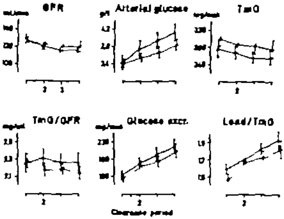


Fig. 3. GFR, arterial glucose concentration, TmG, urinary glucose excretion, TmG/GFR ratio and tubular glucose load/TmG ratio during the 4 consecutive clearance periods. The figures show the mean values and the standard error of the mean. ▲——▲ = group A, ○– –○ = group B.

RESULTS

In group A the mean value of GFR was 102 ml/min (range 73–129 ml/min) and in group B 115 ml/min (range 93–134 ml/min). The mean values of TmG (mg/min) were 265 ± 15 in group A and 288 ± 18 in group B. This difference was not significant. In Fig. 4 TmG has been plotted against GFR and the *regressions have been calculated.* There were significant positive relationships between TmG and GFR in both groups, but no difference in the levels of the regression lines was observed.

In the total material (group A + group B) the following regression of TmG (y) on GFR (x) and mean serum calcium (z) was obtained:

$$y = 173.57 + 2.83x + 20.53z$$
$$= 0.81 \ (p < 0.01)$$
$$r = -0.02 \ (p > 0.1).$$

Thus there was a significant relationship between TmG and GFR, but no relationship was found between TmG and mean serum calcium level. *Neither was any relationship found between* TmG and maximal serum calcium.

Fig. 5 shows the relation between the mean and maximal calcium levels and the ratio TmG/GFR. A mean calcium level of 5.1 mEq/l and a maximal calcium level of 5.5 mEq/l divide the total material into those with proved hyperparathyroidism and those with assumed normal parathyroid function. The ratio TmG/GFR, however was evenly distributed and both high and low values were recorded in both groups.

The patient who was studied both before and 5 months after the surgical removal of a parathyroid adenoma showed a slight increase of TmG after the operation, and the other patient studied twice showed no change in the TmG.

The phosphate clearance, which was measured during the initial 3 clearance periods, was significantly higher in group A than in group B (19.3 ± 2.3 and 12.3 ± 1.3 ml/min respectively). There was no relationship between TmG and phosphate clearance in either group. The glo-

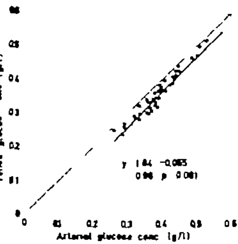


Fig. 2. Correlation between arterial and venous glucose concentrations at elevated blood glucose levels.

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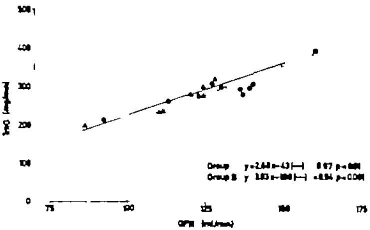


Fig. 4 Correlation between GFR and TmG in group A (▲) and in group B (● = normal *parathyroid glands found at exploration*, ◐ = no exploration performed, normal serum calcium concentration, ○ = postoperatively after removal of adenoma).

merular filtration rate increased on an average 10% during glucose infusion periods compared with the initial periods.

DISCUSSION

The studies on tubular reabsorption of glucose in hyperparathyroidism made by Halver (5) were initiated by the finding that there may be a competition between phosphate and glucose in the tubular reabsorption (10). Parathyroid-hormone-induced inhibition of the tubular reabsorption of phosphate should form a correspondingly larger part of the transport apparatus available for the tubular transport of glucose.

The present investigation has not demonstrated any significant difference in TmG between patients with hyperparathyroidism and patients with assumed normal parathyroid function, either when comparing the absolute values of TmG or when comparing TmG in both groups at equal values of GFR. No relationship has been observed between TmG and serum calcium. Thus the results in the present investigation give no support for the opinion that a determination of TmG could be of value for the diagnosis of hyperparathyroidism.

Although the patients in group B do not constitute an ideal normal material, it cannot seriously be argued that the majority of them had hyperparathyroidism and should have been operated on. All had lower mean serum calcium levels and lower phosphate clearance than the patients in group A. In addition, the two cases who were investigated twice showed a stable ratio, although one had an adenoma removed and the other a normal parathyroid gland removed.

In Halver's investigation (7) the TmG has been related to GFR by using the ratio TmG/GFR. According to Halver this ratio was used because differences in TmG should be corrected for differences in the mass of functioning nephrons. This ratio was increased in hyperparathyroidism. Our results, however show an even distribution of

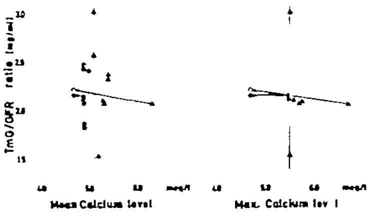


Fig. 5 Correlation between mean and maximum serum calcium levels and TmG GFR ratio in group A and in group B. Symbols as in Fig. 4. The arrow combine the observations in the two patients which were studied twice.

high and low values in the two groups, and no difference in this ratio between the groups could be observed.

For comparing the results in the present investigation with those obtained by Halver (7) the regressive relationship between TmG and GFR has been estimated with the values for these parameters presented by Halver. As was found in the present investigation, Halver's material shows positive and significant regressions between TmG and GFR, both in the group with hyperparathyroidism and in the control group. In contrast to the results in the present investigation, the regressions are significantly different in Halver's material, i.e. TmG is significantly higher in hyperparathyroidism when compared at equal values of GFR.

Our results are then at variance with those obtained by Halver (7), which is difficult to explain. The present material mostly contained cases with hyperparathyroidism, without pronounced hypercalcaemia, and a well preserved renal function. It may be that the specific effect of parathyroid hormone on the tubular reabsorption of glucose as observed by Halver is more easily revealed when the renal function is impaired. Thereby the aid in the differential diagnosis in hyperparathyroidism with slightly increased serum calcium level is missed. However, it is of interest to note that also in our material the patients with proved hyperparathyroidism had a higer TmG at equal values of GFR than the controls, but not to a statistically significant degree.

Halver has also noted a considerable overlap, amounting to at least 15% false negative values. He has also observed a few cases with very low values for the TmG/GFR ratio in proved hyperparathyroidism. Whether these low values are found in a different type of hyperparathyroidism, as suggested by Halver, or appear merely as a chance variation cannot be settled for the moment. We are inclined to favour the latter possibility.

The phosphate clearance was on an average higher in the cases with proved hyperparathyroidism, but the splay of values was considerable in this study as in many others, which invalidates the reliability of this test. At least partly the splay of values is due to neglected standardization of the test concerning the phosphate intake, a point stressed by Reiss (11). The data on the mean phosphate clearance are included here only as additional evidence that cases in the control group did not suffer from hyperparathyroidism.

A measurement of the calcium level retains its position as the most reliable index of parathyroid function when other causes of hypercalcaemia have been excluded.

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KIDNEY PRESERVATION FOR 24 HOURS AFTER ONE HOUR OF WARM ISCHEMIA

PRELIMINARY REPORT

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When the warm ischemic period in donor kidneys (the time from clamping the renal artery until the start of the hypothermic perfusion) is only a few minutes, different methods have proved effective in 24-hour preservation (1–4). Results when the warm ischemia is prolonged over 15 minutes or when an agonal phase of the same order of magnitude is present are unpredictable and unsatisfactory (2, 5), an important consideration when transplantation with necro-kidneys is contemplated.

Vasospasm in the agonal phase has been implicated as a factor in failure of kidney transplants (2). Rabbit kidney perfusion studies have shown a marked increase in the vascular resistance (3–4 times) after warm ischemia of one hour even with vasodilators in the perfusate medium (6). Administration of chlorpromazine and heparin intravenously before the warm ischemic period has been shown to reduce the resistance significantly and only minor renal damage followed an ischemic period of one hour (3).

MATERIAL AND METHODS

Ten female Danish Landrace pigs, between 4 and 5 months old, were studied. Anaesthesia was induced by phencyclidinum NFN (Sernylan®), 2 mg/kg b.wt., and maintained with halothanum NFN (Fluothane®) in semiclosed system. The right kidney and ureter were dissected completely and the ureter cut, so that the only remaining connections between kidney and animal were the renal artery and vein.

The animals were divided into two groups. Group 1 (5 animals). The animals were given i.v. infusions of 500 ml 10% mannitol and 1 000 ml normal saline to which was added heparin (5 000 I.U. 10 kg b. wt.) and chlorpromazine (4 mg/kg b.wt.). After this the artery was clamped and the kidney was left undisturbed "in situ" for one hour. It was then removed, flushed with 200–300 ml cold Collins C_4-solution (5°C) to which papaverine 5 mg% was added, and then stored at 0–2°C for 24 hours. Left-sided nephrectomy and autotransplantation were then performed. The transplant function, reflected by serum creatinine values, was followed for one month.

Group 2 (5 animals). The same conditions as in group 1 with the exception that administration of chlorpromazine was omitted before induction of the warm ischemic period. The transplant function was followed until the death of the animals in four cases and for one month in one case.

RESULTS

All animals in group 1 survived. Serum creatinine values from day 1 to day 30 postoperatively are shown in Fig. 1. It will be seen that serum creatinine fell below 20 μg/ml within 14 days after transplantation. Four animals in group died of uraemia within 8 days after transplantation. One animal survived. In this case serum creatinine increased to 210 μg/ml on the 6th day after transplantation and then fell to the lowest value, 17 μg/ml, .5 days after transplantation.

DISCUSSION

At the present time we can say that, following preparation of the animals with heparine and chlorpromazine, the pig kidney can well tolerate warm ischemia for one hour. The use of chlor

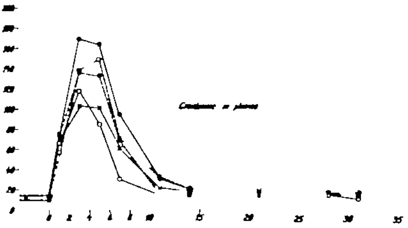


Fig 1 Concentration of creatinine in plasma 0-30 days after transplantation. Ordinate: creatinine in plasma, µg/ml. Abscissa: days after transplantation.

promazine is important, since four animals died when this drug was omitted. Collins solution has proved an effective storage medium under these conditions.

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DIAGNOSIS OF SECONDARY GENERALIZED AMYLOIDOSIS BY FINE NEEDLE BIOPSY OF THE SKIN

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Abstract. A complementary method (fine needle biopsy) for the diagnosis of generalized amyloidosis is suggested, based on the experience of one case and six healthy control subjects.

The diagnosis of generalized amyloidosis offers diagnostic problems in many instances. The disease is often not suspected during life. Since the 1960's rectal biopsy has most frequently been used as method for obtaining histological confirmation of the presence of generalized amyloidosis (3). The rectal biopsy has a great diagnostic accuracy but is unpleasant for the patient.

It has been shown recently that skin biopsy can be used for obtaining the diagnosis of generalized secondary amyloidosis (6) and we therefore studied the possibility of using fine needle biopsy of the skin for this purpose.

CASE REPORT

The patient is a 40-year-old woman who for 11 years has suffered from rheumatoid arthritis, but with negative acryl-test during the whole period. Ten years after the start of the arthritis creatinine in serum was found to be elevated to 3–4 mg/100 ml. There was also a massive albuminuria. Amyloidosis was suspected and a rectal biopsy was performed, which confirmed the diagnosis. Amyloid deposits were found in the media of many small submucous vessels and this led to the conclusion that the patient had acquired generalized amyloidosis, probably of secondary (pararheumatic) type.

Fine needle biopsy from the skin was performed using an ordinary Franzén instrument, i.e. an airtight disposable syringe connected to a specially designed one-hand grip apparatus. The outer diameter of the aspiration needle was 0.6 mm and its length 30 mm (2). Aspirations from the subcutaneous layer just under the corium were performed between the umbilicus and the processus xiphoideus. The aspirates were spread on glass slides. After air drying the preparations were stained with alkaline Congo red (4) and studied by polarization microscopy.

The smears were composed of clusters of fat cells and some fibrous tissue. Only small pieces of glandular tissue and capillary vessels were found. The septa between some fat cells showed a distinct staining with Congo red and bright green birefringence typical of amyloid (Fig. 1).

As controls we used subcutaneous biopsies obtained from the wall of the abdomen of six healthy women between the ages of 25 and 63. The aspirates from these patients did not show any green birefringence.

DISCUSSION

The possibility of employing fine needle biopsy for diagnosis of tumourlike amyloidosis has been described earlier (1). In these cases, however, it is rather easy to obtain appropriate material due to the concentrated masses of amyloid. In generalized, secondary amyloidosis the heaviest deposits are in some internal organs (liver, kidneys, spleen and adrenals). However there is also a widespread manifestation of amyloidosis throughout the body. The skin involvement in secondary generalized amyloidosis is patchy with rings of amyloid around some sweat glands, sebaceous glands, hair follicles and fat cells (5, 6). By aspirating with fine needle in different positions in the subcutaneous layer it is possible to reach rather wide areas, and our work has shown that it is possible to get appropriate material in this way.

Rectal biopsy is a reliable method for the diagnosis of generalized amyloidosis and is often used in cases with a suspicion of this disease. If rectal biopsy is not suitable or cannot be performed, skin biopsy is an alternative method (6).

Fine needle biopsy has many advantages. It is rapid and almost painless for the patient. It can

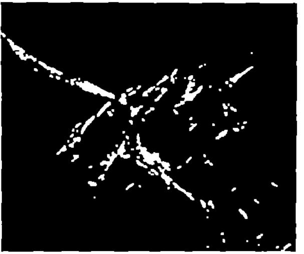


Fig 1 Fat cells and possibly small vessel with deposits of amyloid. Alkaline Congo red. Polarization microscope. Approx. 85

be repeated on the same patient several times without harm. It remains to be demonstrated how reliable the method of fine needle biopsy is, compared to rectal biopsy or punch biopsy of the skin.

Since the method causes little discomfort to the patient, it can be used in screening for generalized secondary amyloidosis among patients suffering from rheumatoid arthritis or other diseases often complicated by secondary amyloidosis.

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ORAL CONTRACEPTIVES AND THROMBOEMBOLIC DISEASE

Swedish Experience

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Abstract. An analysis of almost 400 cases of thromboembolic disease in women on oral contraceptives, reported to the Swedish Adverse Drug Reaction Committee during the years (1965) 1966–70, is presented. The risk of getting thromboembolic manifestation may be calculated at 1 : 3 600 in users of oral contraceptives as compared with 1 : 23 000 among non-users and 1 : 340 during normal pregnancy. The mortality risk is 0.9 patient per 100 000 users and year. Details are presented for the 15 fatal cases. It is emphasized that although statistical evidence has demonstrated significant relationship between the use of oral contraceptives and thromboembolic disease, in the individual case we have no way of telling what actually brought about the thromboembolic condition.

Since the first reports of thromboembolism in conjunction with oral contraception (15, 25) hundreds of similar cases have been published or reported to Adverse Drug Reaction Committees in different parts of the world. The first Swedish case of thromboembolism was reported in 1965 (1), rather more than six months after the indication oral contraception had been approved in Sweden for drugs containing oestrogens and progestogens.

As it was a question of a severe disease, sometimes with fatal outcome, which was suspected to be connected with a drug used chiefly by healthy individuals, both the health authorities and doctors and patients naturally were seriously worried. Several epidemiological studies of the incidence of thromboembolism were initiated, both in Sweden and elsewhere, and investigations of the coagulation pattern in conjunction with oral contraception were started. The relation between the use of oral contraceptives and the occurrence of thromboembolic disease is now established, but many bits of the puzzle must be filled in before there can be a total survey of the situation.

In this paper an analysis is made of the material reported to the Swedish Adverse Drug Reaction Committee and of the morbidity and mortality statistics on thromboembolic disease available in Sweden.

MATERIAL AND RESULTS

During the period Oct. 1965 – Dec. 1970, 399 reports of thromboembolic disease, in which the relation—by the Committee—between oral contraceptives and complication was considered probable or possible, were reported to the Swedish Adverse Drug Reaction Committee (established in Oct. 1965). The number of reports per year will be seen from Fig. 1 and the percentual consumption of oral contraceptives among women aged 15–44 years from Fig. 2.

Chiefly deep venous thromboses have been reported. The distribution of different forms of thrombosis is shown in Table I and the duration of medication in Table II. The patients' ages and the age distribution among nearly 4 000 users of the pill in the Östersund area are shown in Figs. 3 and 4.

Diagnostic reliability

Deep vein thromboses. Of the 270 cases 221 (82 %) had been treated in hospital. In 47 (21 %) of them the records contained a note of predisposing disease. In the remaining 174 cases there was no such note. In 51 of them the diagnosis of deep vein thrombosis was confirmed by phlebography, in another 8 cases in conjunction with thrombectomy or autopsy.

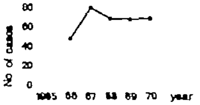


Fig. 1 The annual number of reports to the Swedish Adverse Drug Reaction Committee of thromboembolic disease in women on oral contraceptives.

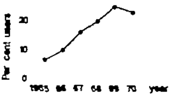


Fig. 2 Percentage of women, aged 15–44 using oral contraceptives in Sweden.

Cerebral thrombosis. The diagnosis was confirmed by angiography in 11 of the 25 cases. In 3 patients the thrombosis was located in the carotid artery in 6 in the middle cerebral artery Other sites were the posterior cerebral, pericallous and vertebral arteries.

Myocardial infarction. On the basis of clinical findings, ECG and laboratory data the diagnosis in the 6 cases—among whom one had also a carotid thrombosis—was considered definite by the reporting physician.

Deaths. Altogether 15 deaths of thromboembolism have been reported in which a connection with oral contraception cannot be ruled out (Table III).

Significance of size of oestrogen dose

In collaboration with the British and Danish Adverse Drug Reaction Committees (11) the material up to 1968 was analysed in respect of the sales of different drugs in relation to the number of reports for each drug. The analysis showed that the risk of thrombosis was dependent on the size of the oestrogen dose (Table IV) but that the type of progestogen might also be of significance. Further analysis of the material should await stabilization of the market following the information on high-dose oestrogen compounds that was issued in Sweden in April 1970.

Significance of blood group

An earlier study has shown that women of blood group O are less susceptible to thrombosis in connection with oral contraception than women in blood group A (14). This difference is also seen in the collected material for the period 1965–70 ($p < 0.001$) (Table V) One third of the patients had been phlebographed. In that group as well the number of patients of blood group O is lower than expected ($p < 0.05$) (χ^2 analysis, performed by Ass. Prof G Eklund, the University of Stockholm.)

Mortality and morbidity studies

For a closer study of the relation between oral contraceptives and thromboembolism, investigations were started in Dec. 1965 of the mortality and morbidity in thromboembolic diseases for the

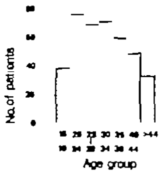


Fig. 3 Age distribution of women contracting thromboembolic disease, while on oral contraceptives, reported to the Swedish Adverse Drug Reaction Committee.

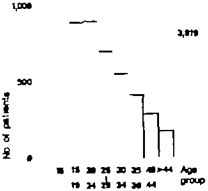


Fig 4 Age distribution among women who during the period Sept. 1 – Dec. 31 1970, bought oral contraceptives in the Östersund area.

Table I. *Localization of thrombotic process in patients using oral contraceptives, reported to the Swedish Adverse Drug Reaction Committee Oct. 1965–Dec. 1970*

	No. of pats.
Deep venous thrombosis (thereof with pulmonary embolism 34)	270
Thrombophlebitis	91
Cerebral thrombosis (including carotid thrombosis)	25
Thrombosis of the coeliac artery	1
Myocardial infarction	6
Thrombosis of the central retinal vein	6
Total	399

period before and after the marketing of oral contraceptives had started. Preliminary results have been reported earlier (29, 30).

Mortality. The official data of the deaths in thromboembolism among men and women aged 15–44 years in Sweden during the period 1961–68 have been received from the Central Bureau of Statistics. The classification numbers (ICD) of diseases covered by the investigation are shown in Table VI.

A comparison has been made between the mortality during the years 1961–63 and 1966–68, i.e. a 3-year period prior and a 3-year period subsequent to the approval of the indication for oral contraception for drugs containing oestrogens and progestogens. It is found that among women the mortality in myocardial infarction has increased in recent years ($p < 0.01$, χ^2 test) (Table VII). For arterial thrombosis the situation is reversed. For venous thrombosis, including pulmonary embolism and cerebral thromboses, no significant differences appear.

For men in the same age group there was an increase of cerebral thromboses ($p < 0.05$) but no change as regards other forms of thrombosis.

Morbidity. In recent years a hospital record linkage scheme has been introduced in the Uppsala region and parts of the city of Stockholm—together representing about one fifth of the population of Sweden. By means of this system the medical records for patients hospitalized because of specified diseases can be retrieved.

A study has been made of women, aged 15–44 years, who during the years 1964–68 (the last year for which complete data are available) had been treated in hospital for some form of thromboembolism (for diagnoses see Table VI.) Data were received concerning some 1 400 patients. Nearly one half were excluded primarily owing to the presence of another simultaneous disease which might cause thrombosis. After perusal of the records of the remaining cases another large group with uncertain diagnoses, complicating diseases which were not diagnostically recorded, etc. was also eliminated. To all other patients an inquiry was sent as to whether they had used oral contraceptives prior to their illness. The results are shown in Tables VIII and IX. The study shows that thromboembolic disease without predisposing factors is uncommon among women of fertile age. An increase has, however, taken place since the introduction of oral contraceptives.

Table II. *Duration of use of oral contraceptives in patients contracting a thromboembolic disease while on oral contraceptives*

Figures within parentheses denote %

Medication time (mo.)	Deep venous thrombosis	Thrombophlebitis	Cerebral thrombosis (including carotid)	Myocardial infarction	Thrombosis of the coeliac artery	Thrombosis of the retinal vein	Total
0– 3	88 (39)	4 (4)	8 (32)		1	2	103 (28)
4– 6	38 (17)	38 (35)	4 (16)				80 (24)
7– 9	16 (7)	23 (21)	2 (8)	1		1	43 (10)
10–12	27 (12)	15 (14)	2 (8)	2		1	47 (13)
13–24	31 (14)	14 (13)	4 (16)	1		2	52 (14)
> 24	28 (12)	15 (14)	5 (20)	2			50 (12)
Total	228	109	25	6	1	6	375[a]

[a] Medication time not adequately reported in all patients.

Table III *Fatal cases of thromboembolism in women taking oral contraceptives*

Year of death	Age (y)	Cause of death	Oral contraceptive: Brand	Oral contraceptive: Steroid content (mg)	Duration of use (mo.)	Notes
1965	30[a]	Thrombosis of the coeliac artery	Lyndiol mite	Mestranol 0.075 Lynoestrenol 2.5	2	
1966	42	Pulmonary embolism	Conluton	Mestranol 0.100 Norethisterone 2	1	
1966	31[b]	Thrombosis of the internal carotid artery	Lyndiol mite	Mestranol 0.075 Lynoestrenol 2.5	6	Headaches after few months on oral contraceptives. Also took ergotamine preparations
1967	27	Pulmonary embolism	Lyndiol mite	Mestranol 0.075 Lynoestrenol 2.5	14	Operated inguinal hernia — pulmonary embolism a few weeks postoperatively
1967	17	Pulmonary embolism	Lyndiol	Mestranol 0.150 Lynoestrenol 5	10	Defective fibrinolysis demonstrated in one brother. Slight "hepatitis" demonstrated at autopsy—considered to be caused by the contraceptives
1967	31	Pulmonary embolism Multiple arterial thromboses	Anovlar Anovlar mite	Ethinyloestradiol 0.050 Norethisterone 4 (mite) 3	24	
1967	43	Pulmonary embolism (recurring)	Unknown	—	"a few"	Chest pains 6 weeks prior to the final embolic episodes
1968	18[c]	Thrombosis of the internal carotid artery	Lyndiol mite	Mestranol 0.075 Lynoestrenol 2.5	18	
1968	[illegible]6	Pulmonary embolism Thromboses of the pelvic veins	Conluten	Mestranol 0.100 Norethisterone 2	18	
1[illegible]	29	Thrombosis of the middle cerebral artery	Anovlar mite	Ethinyloestradiol 0.050 Norethisterone 3	48	Mild essential hypertension
1968	20	Pulmonary embolism	Unknown	—	6	
1968	41	Thromboses of the basilar vertebral and posterior cerebral artery	Unknown	—	36	Mild essential hypertension—several years
1969	22	Pulmonary embolism Thromboses of the iliac veins	Conlumett	Mestranol 0.100 Norethisterone 1	3	Upper respiratory infection 2 months prior to embolism
1969	27	Pulmonary embolism Thrombosis of the femoral vein	Unknown	—	2	
1970	34	Pulmonary embolism of the iliac veins	Conluten followed by Conlumett	Mestranol 0.100 Norethisterone 2 Mestranol 0.100 Norethisterone 1	18	

Four cases have been published previously by [a] Wehn and Persson (28), [b] Brohult et al. (5), [c] Ask-Upmark et al. (2).

Table IV *Comparison of relative risks of venous thromboembolism in the United Kingdom, Sweden and Denmark (11)*

Values based on an expected number of less than 10 reports are shown in italics

	Oestrogen dose (μg)				
	Mestranol			Ethinyl-oestradiol	
	150	100	75–80	50	No. of reports
United Kingdom	2.4	1.6	1.2	1.0	780
Sweden	7.3	1.6	1.4	1.0	183
Denmark	3.4	1.6	0.9	1.0	122

It must be pointed out that the original material was large and the finally analysed material small. This was due to our desire to study, as far as possible, the effect of oral contraceptives alone.

A statistical analysis has been made of the *deep venous thromboses*, the only group which was sufficiently large for analysis. Although an increase of this form of thrombosis has taken place in the general population (Table IX), the increase among users of the pill is greater than among non-users, which suggests an increased risk of thrombosis through the use of the pill ($p < 0.001$ – modified χ^2 test).

Risk figures

On the basis of the figures in the morbidity survey for the Uppsala region, in which the number of women is defined, and assuming that the use of the pill in the Uppsala region in 1968 averaged 20%, the incidence of thrombosis among users of the pill may be calculated at 1 : 3 600 and among non-users 1 : 23 000. By way of comparison it may be mentioned that the risk of thrombosis during normal pregnancy—calculated in the same region and during the same period—may be estimated at 1 : 340.

The number of *deaths* which may be related to oral contraceptives was 15 during the period 1965–70. This would represent an average mortality of 0.9 per 100 000 users per annum (Table X). A perusal of all hospital records of patients who according to the Register of Deaths had died of thromboembolism in 1968 and those who in the same year were reported to the Adverse Drug Reaction Committee, showed that all deaths which could be suspected to be connected with use of the pill had been reported to the Committee.

The risk of death from pulmonary embolism or cerebral thrombosis in women on the pill has been shown to increase with age (10). Similarly it can be shown from our data that the incidence of thromboembolism increases with age (Fig. 5).

DISCUSSION

It was not until 1967 (4) that enough statistical evidence was collected in the United Kingdom to show a correlation between the use of oral contraceptives and death from pulmonary embolism or cerebral thrombosis in the absence of predisposing conditions. The mortality from these two diseases attributable to the use of oral contraceptives by healthy women was estimated at 1.3 per 100 000 users aged 20–34 and 3.4 per 100 000 users aged 35–44 per annum (10). In non-users the mortality was 0.2–0.5 per 100 000. The risk of hospital admission for venous thromboembolism was

Table V *Blood group distribution among women who developed deep venous thrombosis when using oral contraceptives (hospitalized patients without predisposing disease)*

Figures … than parentheses denote

	Blood group				
	A	B	AB	O	Total
Thromboses in patients on oral contraceptives	65 (60)	15 (14)	9 (8)	20 (18)	109 (100)
Whereof with positive phlebogram	20 (61)	6 (18)	3 (9)	4 (12)*	33 (100)
Controls	7 108 (45)	1 759 (11)	887 (6)	6 048 (38)	15 802 (100)

$0.01 < p < 0.05$ $p < 0.001$ χ^2-analyses—significance of deviation from controls indicated by p-values.

Table VI. *Diseases included in mortality and morbity study*

ICD numbers (revision 1965)	Diagnosis
289.42	Infarctus lienis
433–434	Embolia et thrombosis cerebri
435–438	Morbi vasorum cerebri et medullae spinalis alii s. male definiti
377.02	Embolia arteriae centralis retinae
377.03	Thrombosis venae centralis retinae
410	Infarctus myocardii
444	Embolia et thrombosis arterialis
451	Phlebitis et thrombophlebitis extremitatum inferiorum
453	Phlebitis et thrombophlebitis loco alio indicato
450	Embolia et infarctus pulmonis
453	Thrombosis venarum
444.20	Embolia mesenterii
444.20	Thrombosis mesenterii
444.30	Infarctus renis
444.30	Embolia renis

estimated to be 1 2 000 for women using oral contraceptives compared with 1 20 000 for women not using them (27).

The British findings regarding venous thrombosis have been confirmed later in American studies (17 23 26). For myocardial infarction

Table VII. *Mortality in thromboembolic disease in Sweden, men and women, aged 15–44 years*

Figures within parentheses denote number of deaths per 100 000 in the respective age groups

Differences between time periods analysed by χ^2-test and the significance of the differences indicated by the *p*-values

Diagnosis (with ICD numbers)	Men 1961–1963	Men 1966–1968	Women 1961–1963	Women 1966–1968
	$p < 0.05$			
Cerebral thrombosis (433, 434, 435–438)	16 (1.0)	31 (1.9)	16 (1.1)	21 (1.3)
			$p < 0.01$	
Myocardial infarction (410)	265 (17.0)	271 (16.7)	25 (1.6)	50 (3.2)
			$p < 0.05$	
Arterial thrombosis (444)	5 (0.3)	2 (0.1)	8 (0.5)	1 (0.1)
Deep venous thrombosis (pulmonary embolism included) (450, 451 453)	13 (0.8)	23 (1.4)	12 (0.8)	23 (1.5)

and thrombosis in the vessels of the eye there is not enough statistical evidence definitely to prove or disprove a cause–effect relationship with oral contraceptives (7 16).

However Oliver (21) found a higher than estimated prevalence of oral contraception among young women with myocardial infarction. He suggested that oral contraceptives on their own do not increase the risk of thromboembolism but that they might do so in women otherwise prone to ischaemic heart disease.

Our results show an increase in morbidity in venous thromboembolism among women on oral contraception (Tables VIII and IX). The *morbidity* figures seem to be of the same order of magnitude in Sweden, the United States and the United Kingdom. The *mortality* figures, on the other hand, seem—for unknown reasons—to be lower in Sweden than in the other countries (0.9 compared to 3.6 and 3.3 respectively per 100 000 women and year).

We have found an increase in the mortality from myocardial infarction among women aged 15–44 in Sweden (Table VII). We have no knowledge concerning the use of oral contraceptives in that group. A detailed study has been started.

It must be emphasized that the accuracy of the diagnosis varies in our material. As has been pointed out, only patients in whom the connection between the use of oral contraceptives and the

Table VIII. *Morbidity in thromboembolism in the Uppsala region and a Stockholm area with together approximately 1.5 mill. inhabitants—300 000 women, 1964–68. Women aged 15–44 years hospitalized because of thromboembolism without predisposing illnesses*

Figures within parenthesis denote patients known to have been on oral contraceptives when taken ill

Diagnosis	1964	1965	1966	1967	1968
Cerebral thrombosis			2	4 (2)	6 (3)
Myocardial infarction	2	2 (2)	2		
Deep venous thrombosis	5 (1)	10 (8)	13 (8)	28 (20)	28 (18)
Retinal vein thrombosis		2	2	1	3 (1)
Coeliac artery thrombosis				1 (1)	
Total	7 (1)	14 (10)	19 (8)	34 (23)	37 (22)

Table IX. *Morbidity in venous thromboembolism in the Uppsala region and a Stockholm area 1964–68. Women aged 15–44 years hospitalized because of venous thromboembolism [illegible]ithout predisposing illnesses*

Year	No. of pats. using oral contraceptives (expected no. [illegible] parentheses)[a]	Significance of difference between observed and expected cases[b]	Not on oral contraceptives	Oral contraceptive medication unknown	Total	[illegible] oral contraceptive users in population
1964	1 (0.05)	$p<0.001$	4		5	1
1965	8 (0.70)	$p<0.001$		2	10	7
1966	8 (1.30)	$p<0.001$	3	2	13	10
1967	20 (4.48)	$p<0.001$	7	1	28	16
1968	18 (5.60)	$p<0.001$	10		28	20

[a] Calculated as the product of the two last columns, i.e. as if no difference with respect to the morbidity between oral contraceptive [illegible] users and the remaining women aged 15–44 years existed.
[b] Tested by means of the Poisson distribution.
Calculated from the sales on the assumption that all the tablets sold were consumed and used for 13 cycles per year.

thromboembolic disease was evaluated as probable or possible by the Swedish Adverse Drug Reaction Committee ha[illegible]e been included in the study. The percentage of women in whom angiographic evidence was present has been given (see Material and results). It [illegible]aries from one form of thrombosis to another.

The mechanism by which thromboembolism occurs during oral contraception is not known. It is probable that the oestrogens play a major role. Several authors (6, 13[illegible]18) have shown an increased incidence of puerperal thromboembolism after suppression of lactation with oestrogens. These substances have also been incriminated as a cause of thromboembolism in controlled clinical trials of their value in coronary artery and cerebrovascular disease (22, 24). An increased risk has also been reported among men treated with oestrogens for prostatic cancer (3).

Further evidence in this direction has been presented by the Adverse Drug Reaction Committees in the United Kingdom, Sweden and Denmark, which ha[illegible]e in[illegible]estigated the possible differences in the risks associated with the [illegible]arious preparations (11). A positive correlation between the dose of oestrogen and the risk of pulmonary embolism, deep vein thrombosis, cerebral thrombosis and coronary thrombosis was found in the United Kingdom. A similar association was found for venous thrombosis and pulmonary embolism in Sweden and Denmark. No significant differences could be detected between sequential and combined preparations containing the same doses of oestrogen, nor between the two oestrogens, ethinyloestradiol and mestranol.

Table X. *Deaths in connection with the use of oral contraceptives*

The Swedish Adverse Drug Reaction Committee was established in Oct. 1965. It is thus difficult to estimate risks for 1965. If the figures for 1965 are excluded, the total number of deaths is 13 in 5 years or 0.9 per 100 000 and year

	No. of pats.	Deaths per 100 000 users and year
1965	2	1.9
1966	1	0.7
1967	3	1.2
1968	6	1.9
1969	2	0.5
1970	1	0.3
Total	15	0.9

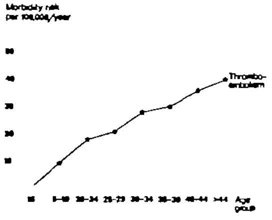


Fig. 5. Calculated risk of thromboembolic disease in various age groups while on oral contraceptives.

Following this report a comment was issued on high-dose oestrogen preparations. This was followed by a change in the prescribing habits in the direction towards low-dose oestrogen preparations.

There have been a multitude of reports, many conflicting, concerning the changes of coagulation factors in oral contraceptive users. Most of the studies demonstrate an increase in factors II, VII and X, and factor VII–X (P & P) complex, with the use of oral contraceptives (31). There is no decrease in fibrinolytic activity as seen in pregnancy Most authors agree that these elevations in coagulation factors do not predispose to venous thrombosis. The changes can be induced by oestrogens alone, but not with progestogen (9)

An interesting finding is that some factors involved in blood clotting and fibrinolysis increase during treatment with oral contraceptives (31) Approximately in the middle of each period of treatment there is a maximal concentration of fibrinogen, plasminogen and spontaneous fibrinolytic activity which then successively decrease towards the end of the tablet period. This may lead to a periodic imbalance in the haemostatic mechanism. If during this period, other factors predisposing to thrombosis are present, the changes in blood coagulation and fibrinolysis may be the factors which cause the thrombosis.

Lately a special interest has been directed to the activators of fibrinolysis which are normally located in the vessel wall from which they can be released. Nilsson (19) and Nilsson and Åstedt (20) have shown that high doses of oestrogen given to women in the menopause induce a marked rise of fibrinogen and plasminogen in plasma. Concomitantly there is a significant decrease of the amount of activators of fibrinolysis in the vessel. With doses such as those used in oral contraceptives, on the other hand, no inhibition of the fibrinolytic activity was found.

Another interesting observation has been made by Fagerhol et al. (8) who demonstrated a reduced amount of antithrombin III in women on the pill. The finding suggests that women on the pill are in a prethrombotic or hypercoagulable state.

Vascular changes, both in the intima and the media, have been demonstrated by Irey et al. (12) in women who died from thrombosis in connection with the use of oral contraceptives. The authors themselves, however state that further studies are necessary to clarify the possible relationship.

When the cause–effect relationship between oral contraceptives and thromboembolism was proved, investigations were carried out in order to see whether any group of women was especially susceptible to this adverse reaction. Most of the factors that might predispose to venous thrombosis, such as recent surgical operations, smoking, the question of blood groups (vide supra) etc., have also been discussed in this connection. With the exception of the blood groups, no further information on such factors has emerged from this study

It is very important to realize that although *statistical evidence* has demonstrated a relationship between the use of oral contraceptives and thromboembolic disease, in the *individual case* we have no ways of telling what actually brought about the thromboembolic condition. The most we can say today is that oral contraceptives *might* have played a role.

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Coronary Heart Disease in Seven Countries. Edited by Ancel Keys, Ph.D. 211 pp. $6.00. American Heart Association Monograph No. 29 Suppl. I to Circulation, April 1970.

A research team from the International Cooperative Study on Cardiovascular Epidemiology examined 12 770 men aged 40 through 59 years in seven countries—the US, Finland, Italy Yugoslavia, the Netherlands, Greece and Japan. The study groups were made up of geographically defined populations in which more than 95% of men 40 to 59 years old participated.

The examinations were standardized and included questionnaires, physical examinations, ECG exercise tests, and blood studies. Average diets were estimated from surveys on random samples.

After entry examination the men were followed with checks on mortality and major morbidity and re-examined, as at entry after five years. Fewer than 0.5% were lost to follow-up.

The age-standardized average yearly incidence of coronary heart disease (CHD) per 10 000 men was 198 for Finland, 177 for the US, 139 for the Netherlands, 100 for Italy 53 for Yugoslavia, 32 for Greece, and 15–20 for Japan.

The so-called risk factors could not explain differences in incidence between countries. Body weight, body fatness and physical activity were not related to the differences between samples in the incidence of CHD. The groups with the lowest incidence of CHD also had the lowest incidence of hypertension. The cholesterol concentration in blood proved to be the outstanding risk factor. This concentration was closely related to the proportion of calories provided by saturated fatty acids in the diet but only slightly related to the proportion of total fat calories. The incidence of CHD was significantly related statistically to smoking habits in the US but not in the other groups. Deaths from all causes were also correlated with cigarette smoking in the US but not in the others.

Ischemic type of ST depression in ECG after exercise was associated with CHD incidence rate more than double the rate in men without this abnormality.

During the five-year study period there were 588 deaths including 158 from CHD. Among the US men 62 of the total 125 deaths were due to CHD. In Finland 38 of 111 deaths were due to CHD and in the Netherlands 16 of 50. For all other national groups combined only 1 in 8 deaths was due to CHD.

Stefan Hofvendahl, Stockholm

Congress Announcements

Sixth International Symposium on Basic Progress in Blood Transfusion will be held in Brussels, Belgium, February 2, 1972.

Secretariat. International Symposium, Postbus 21 3000 Leuven, Belgium.

An IAEA symposium on Nuclear activation techniques in the life sciences will be held in Ljubljana, Yugoslavia, April 10–14 1972.

Organizers: International Atomic Energy Agency Kärnt nerring 11 13 A 1011 Vienna, Austria.

Scientific secretaries: Dr G. B. Cook and Dr R. M. Parr.

Further information and forms to accompany abstracts of papers intended for presentation at the Symposium may be obtained from national authorities for atomic energy matters. Abstracts must be submitted through these authorities so as to reach the International Atomic Energy Agency before December 13, 1971

The Fundación Viviana Luckham has instituted the *International Prize Fundación Viviana Luckham,* 1972.

The prize is intended to honour report of original research related to blood platelets (morphology physiology biochemistry pathology etc.) and/or their relationship to thrombosis and blood vessels, and to promote communication and interchange between research workers in differ ent parts of the world.

For further information and rules for the 1972 contest, pply to Dr Edgardo S. Sack, Fundación Viviana Luckham, Hospital Juan A. Fernández, Cervido 3356, Buenos Aires, Argentina.

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CORONARY CIRCULATION DURING HEAVY EXERCISE IN CONTROL SUBJECTS AND PATIENTS WITH CORONARY HEART DISEASE

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Abstract. Coronary and systemic circulation has been measured in 10 patients with coronary heart disease (CHD) and in 11 control subjects during 2–3 periods of increasing exercise loads. The highest work load was close to the maximal tolerable exercise level. *In control subjects* the maximal coronary flow during heavy exercise increased to 460% of the resting value. The myocardial $(a-v)O_2$ difference varied considerably inter- and intraindividually with increasing work load, but calculated for the group the $(a-v)O_2$ difference did not change significantly. The increase in myocardial oxygen demand was thus met almost exclusively by an increase in coronary flow. The myocardial oxygen uptake increased fourfold from rest to heavy exercise with a parallel fourfold increase in the BP × HR product, with a linear relationship over the whole range of values, suggesting that the product is a good index of myocardial oxygen uptake. *In CHD patients* the coronary flow response to moderate exercise was the same as in control subjects. During heavy exercise, however, the coronary flow increase was lower in relation to the BP × HR product than in the control subjects. Coronary vascular resistance did not decrease to the same level as in the controls. The demand for increase in oxygen uptake was met through a compensatory increase in myocardial $(a-v)O_2$ difference, thus making the necessary oxygen delivery possible. During the heaviest exercise, however, a significantly lower myocardial oxygen consumption was recorded in relation to the BP × HR product than in the control subjects. This was interpreted as an inadequate oxygen supply leading to decreased myocardial contractility. The impaired myocardial performance was best demonstrated in two patients with exercise-provoked angina pectoris who developed rapidly increasing left atrial pressure.

The regulation of coronary circulation in coronary heart disease (CHD) is still a matter of considerable controversy.

Experimental studies in animals have shown that with increasing coronary artery obstruction the coronary flow decreases, with subsequent impairment of myocardial performance (14, 37, 44). In man with CHD, measurements at rest have failed to demonstrate a corresponding inadequacy of coronary flow (5, 18, 36). This has been explained by limitations in the clearance methods used for coronary flow determinations (24). An alternative suggestion is that coronary blood flow at rest actually is normal due to various compensatory mechanisms.

The signs and symptoms of myocardial ischemia are rarely seen at rest but are practically always provoked by sufficiently severe exercise or some other stimulus increasing myocardial energy demand. This suggests that the characteristic features of the coronary circulation in CHD ought to be more easily detected when compensatory mechanisms may be expected to be exhausted. The clearance method for coronary flow determinations should not be discarded for this type of investigation until it has been tested under circumstances in which the chances of disclosing a pathological circulatory pattern are optimal.

The present study was designed to investigate coronary circulation in CHD patients and in control subjects during heavy exercise and, by comparison of the two groups, if possible to detect changes in the coronary circulatory pattern in CHD.

MATERIAL

The material consisted of two groups of patients. Group I included patients with typical CHD and group II, the control group, patients without clinically demonstrable CHD. The subjects in both groups were taken consecutively from patients admitted for investigation of chest pain, syncopal attacks or unduly low physical working

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capacity The criteria for CHD were typical clinical signs and symptoms and significant coronary artery stenosis as verified by coronary arteriography using the technique described by Paulin (30). The stenosis was considered significant if the lumen was narrowed by about one half.

The ECG changes during exercise, previous infarctions and other pertinent clinical data are shown in Table I. The patients in the control group had no typical complaints. Their coronary arteriograms showed no significant stenosis. Patients were excluded only if there were specific contraindications such as advanced age, significant valvular heart disease, arrhythmias, marked hypertension, constrictive pericarditis, all factors that could tend to interfere with the assessment of the effect of CHD on the hemodynamic variables measured. The arbitrarily chosen limit for hypertension was resting diastolic cuff pressure of 100 mmHg. With this definition a few patients with moderate hypertensive cardiovascular disease were included especially in the CHD group.

Group I, patients with CHD consisted of 10 patients (8 males and 2 females), mean age 45.3 years, range 29–58 years. Group II, the control group, consisted of 11 patients (8 males and 3 females), mean age 38.3 years, range 27–58 years. All relevant anthropometric data are shown in Table I.

METHODS

The patients were investigated in fasting state, without premedication and in the supine position. They were informed about the aim of the investigation and the procedure of the study and had given their consent to it. The catheterization technique, the equipment used and the method for the determination of coronary flow have been described previously (18). Coronary vascular resistance was calculated by dividing mean arterial pressure by coronary flow and was expressed in mmHg/ml/100 g min. The right atrial pressure was not subtracted from the arterial pressure, since it was uniformly low. Oxygen saturation in arterial, pulmonary artery and coronary sinus blood and oxygen capacity were measured by methods described previously (6). Lactate concentration in arterial and coronary sinus blood were analysed according to the method of Barker and Summerson (1). The patients performed exercise in the supine position on an electrically braked bicycle ergometer (Kifa).

Coronary angiography was performed according to the method described by Paulin (30). The findings were classified from 1–5 according to Paulin (Table I). The classification was applied to the three major branches. Pathological collateral network was also noted.

PROCEDURE

All patients were investigated at 2–3 work load levels, the highest load being as near the maximally tolerable work load as possible. This load was selected individually for each patient on the basis of a previous exercise test. The lower loads were chosen to cover the widest possible range of loads. In some patients the first study was done at rest. Each procedure took 14–18 min and the patient rested for 15 min between each work loads. After 5 min exercise the measurements started with pressure recordings. Coronary blood flow was determined and at the same time the cardiac output was measured. New pressure recordings were taken and final blood samples for the calculation of the arteriovenous difference of oxygen and lactate were drawn. ECG was recorded continuously and heart rate was calculated simultaneously by each of the above-mentioned procedures. The second pressure recording during each procedure was used for the calculations. The heart rate value used was the mean of a number of recordings from the time of coronary flow determination to the final blood sampling during each procedure. However for the correlation between heart rate and coronary flow the simultaneous heart rate recording was used for calculation.

Statistical methods

Relationships which were not obviously curvilinear were approximated with straight lines.

When an individual effect was expected, observations from different individuals could not be pooled for calculation of regression lines. In these cases the regression line for each individual was calculated and used as a characteristic.

When the relationships were obviously curvilinear the patients were compared with the aid of interpolated values of the dependent variable for given values of the independent variable. The level of significance was 5%.

RESULTS

All data for both groups are presented in Table II

The results of exercise testing prior to the present investigation are shown in Table I. During the investigation the individually chosen maximal exercise level was sufficient to cause chest pain in two patients (nos. 6 and 9) and exhaustion in the remaining patients of the CHD group. In the control group all subjects experienced either general fatigue or fatigue in the legs. The maximal work load used during the investigation varied in the CHD group between 200 and 900 kpm/min with a mean of 400 kpm/min and in the control group from 300 to 900 kpm/min with a mean of 605 kpm/min.

Relationship between heart rate and work load

Both groups increased to high heart rates at the highest work load. The higher maximal heart rates in the control group reflected the generally higher work loads in this group. The heart rate response to exercise did not differ significantly between the groups. This was true both for the

Table I. *Anthropometrical and clinical data for patients with CHD (nos. 1–10) and control subjects (nos. 11–21)*

Function group classifications according to N.Y. Heart Ass. Coronary arteriography findings are classified according to Paulin from 1 to 5 (1 = no demonstrable vessel changes, 2 = irregular vessel walls where no conspicuous decrease in vessel caliber could be seen, 3 = stenosis without delayed filling where the vessel lumen was narrowed by about one half, 4 = stenosis with delayed filling—filling of the vessel segments distal to the narrowing was delayed, 5 = occlusion). RCA = right coronary artery, LC = left circumflex coronary artery, LAD = left anterior descending coronary artery.

Pat. no	Clinical diagnosis	Sex	Age (y)	Height (cm)	B. wt. (kg)	BSA (m^2)	Heart volume (ml)	Heart volume (ml/m^2 BSA)	Resting BP	Effort angina	Function group	Previous infarction	Cor. angio RC	Cor. angio LC	Cor. angio LAD	Comments	Rest ST depr. (>1 mm)	Rest T inversion	Exercise test Max. work load (kpm)	Exercise test Heart rate	Exercise test ST depr. (>1 mm)	Exercise test T inversion	Reason for stoppage Chest pain	Reason for stoppage Dyspnea	Reason for stoppage Fatigue	Reason for stoppage ECG change	Reason for stoppage High heart rate
1	CHD	♂	45	165	60	1.65	740	390	165/95		III	–	3	3	5	Collat.		–	600	138	+	–	+	–	–	–	–
2	CHD + Hypertension	♀	54	161	66	1.71	890	530	130/80	+	II		2	3	2	–	+	+	400	130	+	+	–	–	+	–	–
3	CHD + Hypertension	♂	58	164	75	1.80	680	380	130/90	+	II III	+	2	2	5	Collat.	–		600	146	+	–	–	–	+	–	–
4	CHD	♀	49	172	70	1.82	630	350	140/80	+	II		4	1	5	Collat.		+	400	125	+	+	–	–	+	–	–
5	CHD	♂	48	168	78	1.88	840	450	120/80	(+)	I		2	2	4	–	+		600	140	+	+	–	–	–	+	–
6	CHD + Hypertension	♂	35	181	87	2.06	1030	500	160/95		I		2	2	5	–		–	900	150	+	–	+	–	–	–	–
7	CHD St. post M.I.	♂	36	186	82	2.06	620	300	140/85		I	+	1	1	4	Collat.			900	180	+	–	–	–	–	–	+
8	CHD + Hypertension	♂	29	166	66	1.73	800	480	140/90		II	+	2	2	3	–			900	167		–	–	–	+	–	–
9	CHD	♂	45	167	74	1.82	700	390	140/90	+	II		4	3	3	–			600	140	+	+	+		–	–	–
10	CHD	♂	54	168	74	1.83	1080	570	140/80	+	II		5	2	1				Data missing								
11	Atypical chest pain	♂	36	169	66	1.75	550	320	120/80										900	180	–			–	–	–	+
12	Syncope attacks	♂	29	181	89	2.00	700	330	120/85				1	1	1	–			1200	180					+	–	+
13	St. post myocarditis	♂	27	185	71	1.92	980	500	130/80	–			1	1	1				Data missing								
14	Atypical chest pain	♀	48	157	63	1.63	530	340	120/70				1	1	1	–			600	170	+		+	–	–	+	–
15	St. post pericarditis	♂	27	178	74	1.90	600	310	130/80				1	1	1	–			1200	170				–		–	+
16	St. post myocarditis	♀	48	162	58	1.61	490	300	110/70				1	1	1	–	+		200	107	+	+		–	+	–	–
17	Atypical chest pain	♀	48	164	55	1.57	550	340	125/80				1	1	1	–			800	164	+				+	–	–
18	Atypical chest pain	♂	46	179	86	2.05	800	390	120/80				1	1	2	–		–	900	155			–	–	–	–	–
19	Atypical chest pain	♂	28	166	73	1.80	670	380	120/80	(+)			1	1	1	–		–	900	162		–	–	–	–	–	–
20	Atypical chest pain	♂	45	180	92	2.11	790	380	140/85	–			1	1	1	–		–	600	160		–	–	+	–	–	–
21	Hypertension	♂	39	177	73	1.89	870	450	140/90				1	1	1	–			1200	180	+	+		–	–	–	+

Table II. *Hemodynamic and laboratory data in patients with CHD (nos. 1–10) and in control subjects (nos. 11–21)*

R = rest, W = work, HR I = mean value during investigation procedure, HR II = value recorded simultaneously with coronary flow measurement

Pat. no.	Experimental condition	Work load (kpm/min)	HR (beats/min) I	HR (beats/min) II	Cardiac output (l/min)	Stroke volume (ml)	Blood pressure (mmHg): Aorta or brachial artery Syst.	Diast.	Mean	Left atrium Mean	Coronary sinus Mean	Syst. pressure × HR I/100	Arterial oxygen saturation (%)	Pulmonary artery oxygen saturation (%)	Oxygen capacity (ml/l)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1	R	—	66	66	5.8	88	152	93	115	—		100	97.4		193
	W	150	94	94	9.5	103	177	106	130	—		166	97.2		199
	W	250	106	106	10.1	99	180	105	136	—		191	96.2		195
2	R	—	83	82	4.0	48	170	90	122	—		141	98.0		194
	W	150	107	111	8.0	75	222	100	150	—		238	97.2		205
	W	250	129	129	9.3	72	238	104	164	—		307	96.0		200
3	R	—	71	72	3.8	54	123	73	92	—		87	94.7		175
	W	100	97	95	7.9	81	140	83	109	—		136	97.0		178
	W	200	110	113	9.4	84	137	85	100	—		150	97.3		175
4	W	100	111	113	6.2	55	137	82	105	—	4	152	95.8		187
	W	200	118	127	7.1	57	133	74	99	—	4	157	96.0		185
	W	300	125	130	6.7	51	128	73	99	—	5	160	95.4		183
5	W	200	103	99	10.6	106	159	83	114	8	4	164	97.5		219
	W	430	133	123	13.7	109	185	87	122	8	2	246	97.4		223
	W	600	158	150	14.5	96	201	87	120	4	2.5	318	97.4		228
6	W	200	105	104	12.0	108	176	101	139	5	2	185	98.5	53.7	192
	W	800	149	145	15.6	105	209	115	140	17	3	311	96.8	36.1	194
7	W	300	115	117	11.8	102	1 4	62	78	14	5	143	97.0	54.7	229
	W	600	136	132	14.2	105	134	60	92	16	5	182	96.0	45.3	229
	W	900	150	149	15.4	103	150	75	99	13	7	225	94.0	35.6	231
8	W	200	96	92	11.6	123	154	79	110	7	4	148	96.2	66.4	224
	W	400	112	106	11.2	106	158	71	103	7	3	177	96.3	60.8	217
	W	600	140	128	12.9	99	203	93	122	8	4	287	95.3	53.2	223
9	R	—	78	74	7.8	108	106	55	74	5	4	83	95.4	71.7	175
	W	150	127	126	12.0	97	162	73	102	13	5	206	95.4	55.3	179
	W	300	153	148	13.6	91	162	78	108	22	—	231	93.1	40.5	186
10	R	—	78	78	—	—	124	60	78	3	5	97	94.2	72.2	199
	W	200	108	108	9.7	90	144	67	92	12	5	156	96.1	60.6	200
11	R	—	96	92	8.6	91	144	93	116	—		138	98.3		183
	W	300	142	140	15.5	111	171	103	125	—		243	98.4		186
	W	500	168	163	16.8	98	165	97	128	—		277	99.8		193
12	R	—	56	56	7.7	142	112	73	85	—		62	97.5		196
	W	300	107	107	14.1	132	123	91	102	—		132	97.9		203
	W	600	160	150	19.9	126	123	87	102	—		197	99.6		205
	W	900	195	195	22.8	117	140	96	114	—		273	98.1		206
13	R	—	68	66	8.3	117	125	82	104	—		85	99.2		215
	W	300	97	97	13.6	140	136	101	116	—		132	98.9		216
	W	600	120	128	18.1	131	137	104	120	—		164	98.9		222
	W	900	159	159	22.9	144	147	114	128	—		234	96.4		218
14	W	150	126	121	8.4	68	144	93	113	4	4	181	97.5		191
	W	300	156	146	11.5	74	160	85	108	7	4	250	97.1		191
	W	450	160	155	11.8	75	165	87	117	7	4	264	96.8		190
15	W	200	94	88	10.7	122	156	89	114	9	7	147	97.6	59	175
	W	400	103	98	14.1	141	150	84	105	9	5	155	96.5	50	178
	W	600	123	117	15.4	128	165	85	105	9	4	203	96.6	41	177

Arterio-venous oxygen difference (ml/l) (17)	Oxygen uptake (ml/min) (18)	CF (ml/100 g min) (19)	Coronary vascular resistance (mm Hg/ml/100 g min) (20)	Coronary sinus oxygen saturation (%) (21)	Myocardial arterio-venous oxygen diff (ml/l) (22)	Myocardial oxygen uptake (ml/100 g min) (23)	Arterial lactate (mg/100 ml) (24)	Coronary sinus lactate (mg/100 ml) (25)	Myocardial arterio-venous diff of lactate (mg/100 ml) (26)	Lactate extraction (%) (27)
		75	1.53	34.3	123	9.2	5.7	2.9	2.8	49
		110	1.18	28.8	136	14.9	10.9	6.8	4.1	38
		119	1.14	28.3	133	15.8	17.2	8.6	8.6	50
		67	1.82	38.3	116	7.7	6.1	4.7	1.4	23
		108	1.39	25.5	147	15.9	13.5	12.2	1.3	10
		143	1.15	23.9	144	20.6	23.3	20.3	3.0	13
		62	1.48	29.7	113	7.0	7.1	5.2	1.9	27
		106	1.03	28.9	122	13.0	9.3	9.8	−0.4	−4
		130	0.77	31.0	116	15.0	13.8	12.8	1.0	7
		106	0.99	28.7	130	13.3	68.9	19.8	49.1	71
		124	0.8	23.2	135	16.8	37.9	33.7	4.2	11
		131	0.76	20.2	138	19.2	48.5	43.2	5.3	11
		106	1.08	28.3	152	16.0	12.9	12.1	0.8	6.2
		143	0.85	27.0	157	22.4	40.3	30.6	9.7	24
		163	0.74	23.4	169	27.5	63.8	53.3	10.5	16
82	980	124	1.12	24.1	143	17.7	7.2	6.3	0.9	13
118	1 840	168	0.89	24.6	140	23.5	42.5	39.6	2.9	7
97	1 140	89	0.88	34.5	143	12.9	16.0	10.7	5.3	33
116	1 640	119	0.78	26.1	160	18.8	31.5	19.7	11.8	37
135	2 070	158	0.63	24.8	160	25.3	65.7	53.5	12.2	19
67	775	110	1.00	33.3	141	15.5	1.4	1.8	−0.4	−29
77	860	124	0.83	33	137	17.0	8.2	1.6	6.6	80
94	1 209	178	0.69	33.8	137	24.4	24.4	17.1	7.3	29
42	324	62	1.2	38.6	99	6.2	4.8	4.0	0.8	20
72	862	168	0.61	31.2	115	21.8	14.0	13.4	0.6	4
98	1 334	190	0.57	24.7	127	4.1	28.6	35.1	−6.5	−23
44	—	62	1.26	31.7	125	7.7	3.8	4.4	−0.6	−16
96	689	102	0.9	26.5	139	14.2	8.1	10.5	−2.4	−31
		102	1.14	34.7	116	11.8	1.0	4.2	−3.2	−320
		178	0.7	30.0	127	22.6	16.2	11.6	4.6	60
		228	0.56	32.2	130	29.6	27.3	19.2	8.1	30
		70	1.21	45.7	101	7.1	9.7	5.6	4.1	42
		133	0.77	44.4	109	14.5	10.8	7.5	3.3	31
		178	0.57	40.6	121	21.6	25	19.2	5.8	23
		239	0.44	36.2	128	33.2	60	57.6	2.4	4
		55	1.89	42.0	123	6.8	5.7	6.2	−0.5	−8
		136	0.85	45.3	112	15.2	6.7	6.5	0.2	3
		154	0.78	43.2	124	19.1	6.5	5.2	1.3	20
		184	0.70	32.1	140	25.8	22.1	—	—	—
		143	0.79	44.7	101	14.4	8.1	4.7	3.4	42
		190	0.57	30.0	128	24.3	11.0	5.5	5.5	50
		204	0.57	27.6	131	26.6	33.7	19.6	14.1	42
68	690	106	1.08	35.4	108	11.5	6.6	4.5	2.1	32
83	1 170	106	0.99	26.3	125	13.2	8.3	7.3	1.0	12
99	1 510	139	0.76	25.3	120	17.0	7.6	—	—	—

Table II (*Continued*)

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
16	W	100	107	98	7.5	75	161	81	113	10	0	172	99.4		156
	W	200	110	119	9.2	72	131	62	80	8	−1	144	97.1		156
	W	300	114	127	9.5	77	162	79	106	12	0	185	97.8		131
17	W	200	126	127	12.3	99	150	74	94	4	6	189	98.7	57	154
	W	400	131	148	13.6	93	164	67	93	5	6	248	99.2	45	155
	W	600	158	156	13.7	87	182	73	102	5	4	288	97.6	36	156
18	R	—	68	70	7.6	108	98	45	63	2	1	67	96.7	71	198
	W	400	116	111	13.2	113	132	51	72	3	3	153	96.4	58	197
19	R	—	64	62	7.3	111	120	60	82	6	6	77	96.1	77	189
	W	400	124	119	13.1	116	135	62	77	1	2	167	98.2	53	195
	W	800	142	143	14.7	164	163	65	79	0	2	234	96.9	32	196
20	R	—	73	72	8.0	112	136	76	96	5	1	99	94.7	71	197
	W	300	112	111	14.7	131	164	85	106	4	0	184	94.2	52	198
	W	600	138	132	16.2	121	174	79	104	4	1	240	94.9	41	194
21	R	—	76	76	8.1	104	160	88	118	4	3	122	97.3	75	198
	W	300	113	112	13.0	112	176	97	132	6	2	202	95.9	57	197
	W	600	148	142	17.3	118	180	93	128	3	1	266	93.5	47	198

regression coefficients and for the intercepts at zero work load (Fig. 1).

Relationship between systolic pressure and work load

The systolic pressure rose with increasing work load in both groups. In the CHD group there were a *few* patients with a marked pressure increase on exercise, and as a consequence of this the pressures at the highest work loads were higher in this group than in controls. The increase in systolic pressure for a given increase of work load was significantly higher in the CHD group, while the intercept at zero work load did not differ significantly in the two groups (Fig. 2).

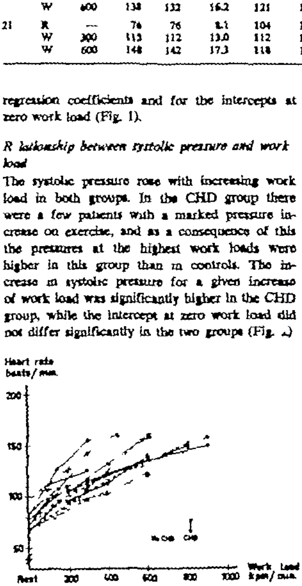


Fig 1 Relationship between heart rate and work load.

Relationship between cardiac output and work load

The cardiac output increase for a given increase in work load in the CHD group tended to be less than in the control group but the differences were not statistically significant (Fig. 3).

Relationship between oxygen uptake and work load

The arteriovenous oxygen difference was measured at each work load in 4 patients in the CHD group and in 6 in the control group. Oxygen up-

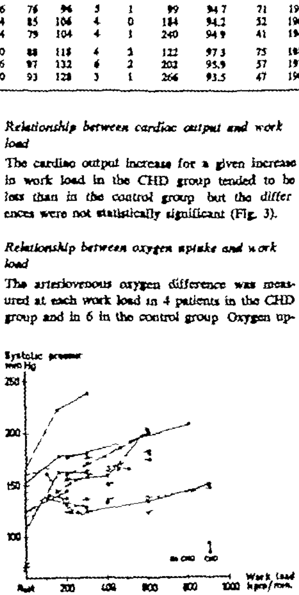


Fig 2 Relationship between arterial systolic pressure and work load.

(17)	(18)	(19)	(20)	(21)	(22)	(23)	(24)	(25)	(26)	(27)
		130	0.87	26.6	113	14.7	8.1	4.2	3.9	48
		158	0.50	24.2	114	17.9	10.6	7.5	3.1	29
		178	0.60	27.4	106	19.0	12.7	10.6	2.1	16
65	790	147	0.64	29.7	107	15.6	14.0	11.7	2.3	16
83	1 135	199	0.49	31.2	105	20.0	31.9	26.6	4.3	20
95	1 320	204	0.50	34.1	99	20.1	49	42.9	6.1	12
51	384	50	1.26	37.8	117	5.8	1.4	1.8	−0.4	29
76	999	110	0.78	40.4	110	12.1	8	1.6	6.6	80
36	265	58	1.41	49.3	89	5.1	3.9	1.7	2.2	56
89	1 156	119	0.65	31.9	127	15.1	6.4	5.3	1.2	19
128	1 878	130	0.60	27.6	136	17.7	58.5	52.2	6.3	11
47	379	78	1.37	32.1	123	8.6	7.4	8.5	−1.1	−15
83	1 210	92	1.15	24.6	138	12.6	10.6	10.6	0.0	0
105	1 703	136	0.76	27.7	130	17.7	17.0	14.5	2.5	15
43	362	93	1.28	32.4	128	11.8	5.3	5.8	−0.5	−9
77	999	168	0.79	37.1	116	19.4	13.2	6.8	6.4	49
92	1 584	260	0.49	35.7	114	29.6	27.2	17.9	9.3	34

take was calculated according to the Fick formula from the values of cardiac output and $(a\text{-}\bar{v})O_2$ difference. The relation between oxygen uptake and work load was approximately linear and in agreement with values published by others (45). There was no significant difference in the oxygen uptake in relation to exercise between the groups (Fig. 4).

Relationship between arterial lactate and work load

Only the maximal values of arterial lactate concentration for each patient were used for the comparison. In the CHD group they varied from 7.3 mg% to 65.7 mg% with mean 33.4 mg% and in the control group from 7.6 mg% to 58.5 mg% with mean 30.8 mg%. The difference between the groups was not statistically significant (Fig. 5).

Coronary Circulation

Relationship between coronary blood flow and heart rate

Coronary flow increased in all patients approximately linearly with increasing heart rate. For the

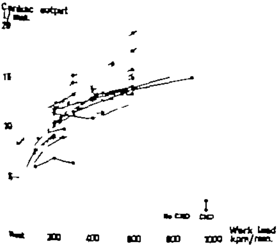


Fig. 3. Relationship between cardiac output and work load.

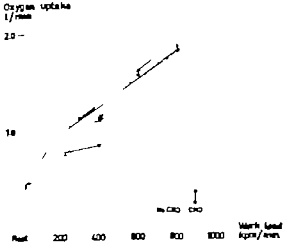


Fig. 4. Relationship between oxygen uptake and work load in the 6 control subjects and 4 patients with CHD in whom oxygen uptake was measured.

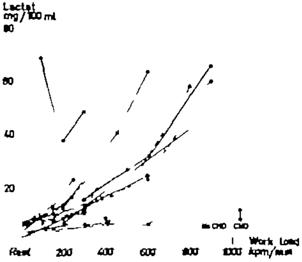


Fig. 5 Relationship between arterial lactate and work load.

6 control subjects and 4 CHD patients in whom measurements were made at rest, the highest flow values for each patient were expressed as the per cent of his resting coronary blood flow The results are shown in Table III. In the control group the maximal recorded flow was 370% of resting coronary flow and in the CHD group 306% of resting coronary flow To estimate the range of coronary flow in normal man, all measurements at rest and during work in the control group were pooled, as no individual effect was expected, and the regression equation was calculated. CF = 1 44 HR − 30.6. From this equation it could be calculated that variation in HR from 60 to 200 beats/min would give a change in CF from 56

Table III. *The maximal recorded coronary flow in percent of resting coronary flow in 4 patients with CHD and 6 control subjects*

Subject no.	Max. CF in % of resting CF
CHD pats.	
1	159
2	213
3	210
9	306
Controls	
11	224
12	370
13	334
19	224
20	194
21	283

to 258 ml/100 g × min, a 4 6-fold variation. The relation between coronary flow and heart rate was not significantly different in the two groups (Fig. 6).

Relationship between coronary blood flow and systolic blood pressure

The blood pressure rose parallel to the increasing coronary flow in all patients but one. In the control group the blood pressure rise was slight. Calculated from regression coefficients of the individual regression equations an increase of coronary flow of 100 ml/100 g × min corresponded to an average increase in systolic blood pressure of 34 mmHg.

In comparing the two groups a tendency for the CHD group to a more marked blood pressure rise for a given increase in coronary flow was found, but the differences between the two groups were not statistically significant (Fig. 7)

Relationship between coronary flow and BP × HR product

At rest and at low levels of BP × HR product, coronary flow values for the control group and

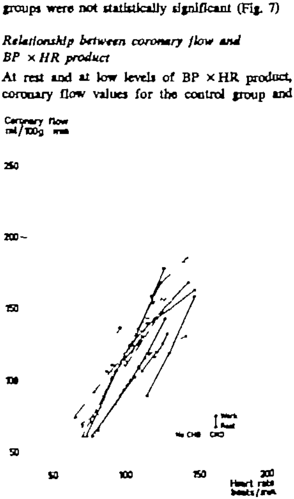


Fig. 6. Relationship between coronary blood flow and heart rate.

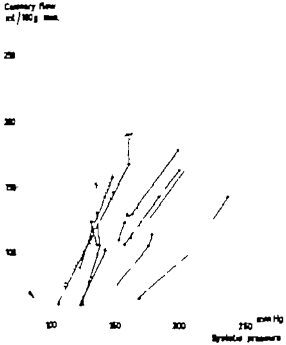


Fig. 7. Relationship between coronary flow and arterial systolic pressure. Symbols as in Fig. 6.

the CHD group were almost identical. With increasing BP × HR values there was an increasing difference between the groups with lower flows in the CHD group. For comparison between the groups the interpolated values of coronary flow at four given levels of BP × HR were used. At the lowest BP × HR level no significant difference could be found between the groups at the highest BP × HR level the coronary flow was significantly lower in the CHD group (Fig. 8)

Relationship between coronary vascular resistance and BP × HR product

At rest and at low levels of work the coronary vascular resistance values were widely scattered both in the control group and in the CHD patients. With increasing work load the coronary vascular resistance diminished in all patients. The control subjects who reached high levels of BP × HR all came close to the same value, 0.5 mmHg/ml/100 g × min. In the CHD group the pattern was more variable. The patients who reached high BP × HR levels also diminished their resistance considerably but when the groups were compared at the highest level of BP × HR the CHD group had a significantly higher coronary vascular resistance (Fig. 9)

Relationship between myocardial (a–v)O_2 difference and BP × HR product

At rest myocardial (a–v)O_2 difference varied widely 89 ml/l – 128 ml/l, both in the control subjects and in the CHD patients without any systematic difference between the groups. With increasing work the myocardial (a–v)O_2 difference decreased in some patients, increased in some and changed first in one and then in the other direction in a few patients, but as a group the

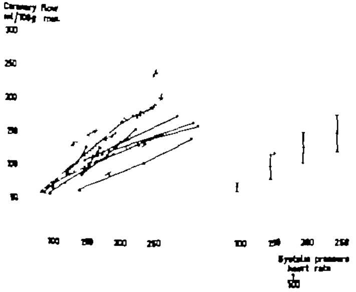


Fig. 8. Left part. Relationship between coronary flow and the product of systolic pressure and heart rate (BP × HR). Right part: Coronary flow values are interpolated to 4 levels of BP × HR. The levels are indicated by small arrows in the left part of the figure. The results are presented as mean values and standard deviations for control subjects and patients with CHD at each level of BP × HR. Symbols as in Fig. 6.

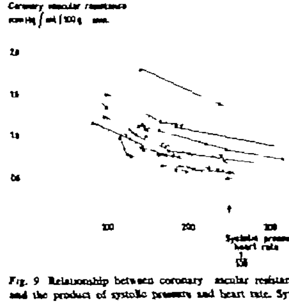


Fig. 9 Relationship between coronary vascular resistance and the product of systolic pressure and heart rate. Symbols as in Fig. 6.

CHD patients showed a greater increase of $(a-v)O_2$ difference. During heavy exercise the highest recorded $(a-v)O_2$ difference in the control group was 140 ml/l, corresponding to an oxygen extraction of 66% and in the CHD patients 169 ml/l corresponding to an oxygen extraction of 76% (Fig. 10)

As can be seen from Fig. 10 in the control group the $(a-v)O_2$ difference changed only slightly while in the CHD patients it increased markedly. Comparing the two groups it was found that at the lowest level of BP × HR product there was no significant difference, while at the highest level the CHD group had a significantly higher myocardial $(a-v)O_2$ difference than the control subjects.

Relationship between myocardial $(a-v)O_2$ difference and coronary blood flow

This relationship is similar to the previous one. The $(a-v)O_2$ difference values are interpolated for five given values of coronary flow and the groups are compared at the highest and lowest of these levels. At the lowest level of coronary flow (70 ml/100 g × min) no significant difference could be found between the groups, but at the highest level (150 ml/100 g × min) the CHD group had a significantly higher myocardial $(a-v)O_2$ difference (Fig. 11)

Relationship between myocardial oxygen uptake and BP × HR product

The myocardial oxygen uptake increased approximately linearly with increasing BP × HR product

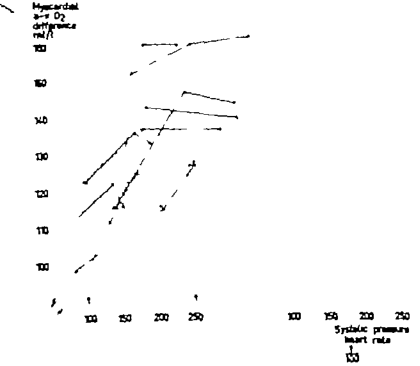


Fig. 10 Left part: Relationship between myocardial arterio-venous difference of oxygen and the product of systolic pressure and heart rate (BP × HR). Right part. Values of myocardial arterio-venous difference of oxygen are interpolated to four given levels of BP × HR. The levels are indicated by small arrows in the left part. Results are presented as mean values and S.D. of control subjects and patients with CHD at each level of BP × HR. Symbols as in Fig. 6.

over the whole range of work levels in the control subjects. An approximate measure of this linearity for each individual subject is the correlation coefficient. In the control group the mean value of the correlation coefficients was 0.98 with a standard deviation of 0.02.

All data of the control group were pooled on the assumption that there was no individual effect and the regression equation for these data was calculated.

$y/1\,000 = 9.9688x - 6...$

x = myocardial oxygen uptake in ml/100 g × min, y = BP × HR product.

The lowest and highest values of BP × HR recorded were 6 800 and 27 100 respectively a fourfold increase. The corresponding values for myocardial oxygen uptake calculated from the regression equation were 7.0 ml/100 g × min and 28 ml/100 g × min respectively also a fourfold increase.

If the interpolated values of myocardial oxy-

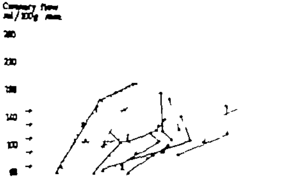


Fig. 11. Upper part: Relationship between coronary flow and myocardial arterio-venous difference of oxygen. Lower part. Values of myocardial arterio-venous difference of oxygen are interpolated to five levels of coronary flow. The levels are indicated by arrows in the upper part. The results are presented as mean values and S.D. for control subjects and patients with CHD at each level of coronary flow. Symbols as in Fig. 6.

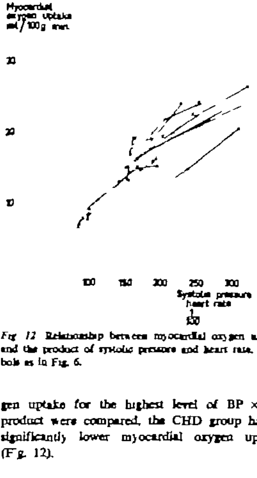


Fig. 12. Relationship between myocardial oxygen uptake and the product of systolic pressure and heart rate. Symbols as in Fig. 6.

gen uptake for the highest level of BP × HR product were compared, the CHD group had a significantly lower myocardial oxygen uptake (Fig. 12).

Relationship between left atrial pressure and BP × HR product

Left atrial mean pressure is an approximate measure of the left ventricular end-diastolic pressure in the absence of mitral valvular disease (4). In this investigation, as has been described previously (17), the left atrial mean pressure changed continuously. It increased uniformly until the third/fourth minute and then fell slowly during the continuous exercise. The values reported here were taken from the final part of the procedure. The only exception from the typical pattern was one of the two patients in whom angina pectoris was provoked (no. 6). He had a normal left atrial pressure at a low work load, but at the highest work load he developed angina pectoris and a high atrial pressure which persisted during the short time work could be continued. The other patient with angina pectoris (no. 9) also had a high left atrial pressure at the highest work load. However he could continue the exercise and the

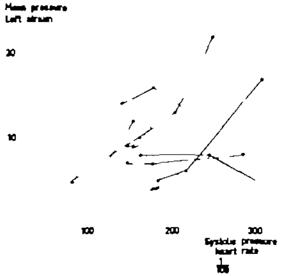


Fig. 13. Relationship between mean pressure in the left atrium and the product of systolic pressure and heart rate in the 8 control subjects and the 6 patients with CHD in whom left atrial pressure was recorded. Symbols as in Fig. 6.

left atrial pressure then decreased slowly as in the other patients, but at a higher pressure level. In the remaining few patients in whom left atrial pressure was recorded, there was no obvious difference between control subjects and CHD patients. In five patients the left atrial pressure decreased with increasing work load, while in six patients it increased slowly (Fig. 13).

Myocardial lactate metabolism

There was no consistent pattern for myocardial lactate metabolism in either of the groups. In most patients the myocardial lactate consumption increased with increasing work load. However, in two patients in the control group the consumption diminished with increasing load and the same was noted in three patients in the CHD group, in two of whom it turned into lactate production (nos. 9 and 10).

DISCUSSION

CHD was defined in this study as arteriographically demonstrated significant stenoses in coronary arteries in conjunction with typical clinical findings. An ideal control group should be identical with the CHD group except for the absence of CHD. This is impossible in practice, since one lacks justification for the present type of investigation in a normal symptom-free population.

In this investigation the CHD patients and the control subjects were taken consecutively from patients admitted for hemodynamic evaluation. This resulted in small age differences between the groups, the mean age in the CHD group being 45 years and in the control group 38 years. The CHD group had a higher blood pressure response to exercise, which was interpreted as part of their vascular disease. This was expected and probably unavoidable because of the close relationship between hypertensive vascular disease and CHD. Regarding the other measured variables, no major differences could be found between the groups. Thus the groups could be considered acceptable for comparison.

The feasibility of the methods

Clearance methods for coronary flow measurements have their limitations. They can only measure average flow. In multicompartment flow systems the regions with slow flow will in many cases, be overlooked and the average flow will reflect mainly the flow of the best perfused areas (23, 24). Therefore the average flow values obtained must be interpreted cautiously and it must be borne in mind that the method will tend to give values that are too high, especially in CHD patients. The question then arises whether this method is feasible in trying to disclose compromised coronary circulation known to exist in CHD (42). Previous investigations have demonstrated that coronary flow at rest and on mild exercise is identical in normal subjects and CHD patients (5, 8, 10, 12, 18, 27, 33, 34, 36). This can be explained by the methodological limitation mentioned. It is, however, also possible that the degree of myocardial oxygen demand, determined for example by the severity of exercise, is of crucial importance in demonstrating the limiting effect of coronary artery stenosis on coronary flow. This effect could be assumed to become apparent when regulatory mechanisms have been exhausted. This reasoning seems to justify the present approach.

The other factor in the calculation of myocardial oxygen uptake, myocardial (a–v)O_2 difference, is based on measurements of oxygen saturation in artery and in coronary sinus blood. Coronary sinus blood sampling has been shown

to be dependent on an accurate technique to avoid right atrial blood admixture. If the sampling catheter is 2 cm beyond the coronary sinus ostium, the risk of right atrial blood admixture is negligible (25). This ideal catheter position was achieved in every case and checked with contrast medium injection. In CHD patients different parts of the venous blood could be assumed to have different oxygen saturations and then the position of the sampling catheter could be critical for obtaining representative blood samples for mean oxygen saturation. Theoretically the catheter could move during vigorous exercise, so that blood could be sampled from different regions at different times. This could not be completely excluded, but the catheter position was checked after each procedure by fluoroscopy and was unchanged. Occasional observations during exercise confirmed the stable position.

Myocardial oxygen uptake, which is a reflection of the energy utilization in the myocardium, is determined by a number of factors of which heart rate, intramyocardial tension and contractile state of the heart probably are the most important (13, 40). Of these factors heart rate is easily recorded. Myocardial tension could not be calculated directly because myocardial wall thickness and left ventricular volume could not be measured. Instead it was estimated indirectly in terms of systolic pressure. Similarly the contractile state of the myocardium could not be measured.

Various indices for myocardial oxygen demand using hemodynamic variables of the type measured in this investigation have their limitations. However the index chosen here, the product of systolic pressure and heart rate, has repeatedly been shown in animal experiments to correlate well with myocardial oxygen uptake (9 19 20, 41). It also has the specific advantage of not requiring the invasive technique, which makes it suitable for wider application. Variations in both cardiac dimensions and contractile states will, however change the relationship between this index and myocardial oxygen uptake.

Coronary hemodynamic pattern in control subjects

At rest the present coronary flow values varied within wide limits as they do in other studies (5 8, 10, 18, 27 31 33 34 36). The maximal *recorded* coronary flow during exercise was 370% of the resting value. The maximal *calculated* coronary flow was 460% of resting coronary flow assuming a change of heart rate from 60 to 200 beats/min. This indicates that the maximal capacity to increase coronary flow is approximately the same in man as in experimental animals (__). No previous studies have shown coronary flow at near maximal work loads in man. During light to moderate exercise the coronary flow in other studies increased in the same fashion as demonstrated here (5 8, 10, 1_, 27 33). In patients with aortic stenosis even the resting coronary blood flow has been shown to be very high because of the sustained high myocardial energy demand (35). Slight physical work in this group of patients caused the coronary flow to increase further reaching approximately the same levels as recorded here.

The main regulating mechanism for coronary flow increase was a decrease in coronary vascular resistance. The resistance at rest and at low work loads varied considerably from subject to subject, but with increasing work loads the pattern became more uniform. In all subjects reaching high work loads the coronary vascular resistance was lowered to approximately the same level, 0.5 mmHg/ml 100 g min. This is in good agreement with animal studies (22) and with measurements on patients with aortic stenosis during moderate work (35). The observed curvilinear approximation to the lowest value might indicate that normal man at near maximal work is close to maximal physiological dilatation of the myocardial resistance vessels.

The myocardial (a–)O_2 difference at rest also varied within wide limits as observed in other studies (7 8 18, 27 33). With increasing work loads the (a–v)O_2 difference increased successively in some subjects and decreased in others. For the whole group there was a tendency to increase with higher work load, even if this increase was not statistically significant. The observed intra- and inter-individual variation of (a–v)O_2 difference may be dependent largely on the error of measurement. However it might also suggest that the regulation of coronary flow is more complex than has previously been assumed. A variation in the fraction of blood flow distributed through a–v shunts would be reflected in such (a–v)O_2 difference changes. Such a–v shunts have been described in man (32). This explana-

tion is to some extent supported by the observations in some subjects that a relatively large decrease of $(a-v)O_2$ difference was accompanied by a markedly increased value of coronary flow and vice versa. A more linear relationship between oxygen uptake and BP × HR was thus obtained than would have been expected from the changes in $(a-v)O_2$ difference. Some subjects obviously met the increasing oxygen demands at high work loads exclusively by increasing coronary flow while others also changed their oxygen extraction. From the regression line of the pooled data it was calculated that the fourfold increase of myocardial oxygen uptake corresponded to a similar fourfold increase in BP × HR product, with a linear relationship over the whole range of values. In this group of normal, or near normal subjects the BP × HR product thus seems to reflect adequately the changes in myocardial oxygen uptake. However the predictive value of this index for the individual subject concerning myocardial oxygen uptake is more limited. Fig. 12 demonstrates that the regression coefficients in the individual regression equations must vary. This individual variation may be due partly to error of measurements and partly to possible changes of cardiac dimensions and contractile state.

Coronary circulation and myocardial metabolism in coronary patients

At low work loads the patients with CHD had coronary flow values in the same range as control subjects. During heavy exercise, however the coronary flow was significantly lower in the patients in spite of a higher driving pressure.

The coronary vascular resistance decreased during exercise in coronary patients, but the decrease was less pronounced and less uniform than in the control subjects. A different response of the vascular resistance to exercise should be expected in a group of patients with CHD in whom the engagement of the coronary arteries varies from single lesions to extensive narrowing of all epicardial vessels. In patients with a sustained high resistance when the stimulus to vascular dilatation is maximal, i.e. heavy exercise, the generalized narrowing of coronary arteries is probably a major flow limiting factor. In this small group of patients with CHD though, no correlation was found between the coronary vascular resistance during heavy exercise and the degree of coronary artery narrowing as estimated from the arteriography.

In a few other investigations coronary flow has been compared in groups of patients with and without CHD during procedures that increase myocardial oxygen demand. In these studies the same increase in coronary flow was found during exercise in both groups. However the moderate amount of stress used in these investigations increased the myocardial oxygen uptake only to a limited extent (5, 10, 26). This is in agreement with our findings that, at low work loads, patients with CHD have the same type of coronary vascular response as control subjects. Maximal or near maximal exercise is thus required to reveal a different flow pattern in patients with CHD.

Myocardial $(a-v)O_2$ difference varied within wide limits at rest in coronary patients. There was no systematic difference compared to the control subjects. This is in accordance with what has been reported previously in man (5, 10, 28, 41). On heavy exercise a wide scatter of values for $(a-v)O_2$ difference was found in the patients with CHD. As a group these patients showed a significantly greater increase in myocardial $(a-v)O_2$ difference than the control subjects. This different response was probably a consequence of the insufficient coronary flow increase in relation to the oxygen demands. The same type of response has been reported from experimental animal studies (38, 39).

Myocardial oxygen uptake increased with increasing BP × HR product in the same fashion as in control subjects on low and moderate work loads. When those myocardial oxygen uptake values that corresponded to the highest values of the BP × HR product were compared, the patients with CHD had significantly lower myocardial oxygen uptake for a given value of the BP × HR product. Similar findings have been reported in animal experiments. Sarnoff et al. (37) found that successive lowering of coronary flow with constant pressures and heart rate resulted finally in decreased myocardial oxygen uptake. This was as a rule combined with signs of left ventricular failure. The results were explained by decreased contractility of the myocardium even if this could not be measured directly in their experiments. Wiggers (43) and Gregg (21) have shown that contractility decreases as a consequence of coro-

nary artery obstructions in dog experiments. The same has been found in man by Gorlin et al. (15), the decrease of contractility being manifested as asynergy of contraction. On the basis of these investigations we interpret our findings of a lower myocardial oxygen uptake in CHD patients at high work loads as secondary to a decreased contractility.

Left atrial pressure was measured only in a limited number of patients. In two patients who developed angina pectoris on exercise (nos. 6 and 9) the left atrial pressure rose to high values, but in the other four in whom it was measured the change in left atrial pressure did not differ from the control subjects. The diminished contractility was thus as a rule not severe enough to cause left ventricular failure as reflected in a rise of left atrial pressure.

The measurement of myocardial lactate metabolism did not give any additional information in this investigation. In two patients with CHD (nos. 9 and 10) the lactate consumption turned into lactate production during heavy exercise, but the rest of the patients behaved like the control subjects. These measurements are of limited value during heavy exercise. Arterial lactate values may vary markedly during continuous work (2). The high and changing arterial lactate values will easily mask small regional changes in myocardial lactate metabolism (3).

It can thus be concluded from this investigation that patients with coronary artery obstructions, in spite of compromised coronary flow, can maintain myocardial oxygen uptake at adequate levels over a wide range of work loads. This is achieved by a compensatory increase of oxygen extraction. When this mechanism becomes insufficient at high work loads, the myocardium responds to the insufficient oxygen supply with decreased contractility.

The increased coronary vascular resistance is probably caused by widespread atheromatous changes. There is a possibility, though, that hypertensive cardiovascular disease, present in some patients, could induce generalized vascular changes in coronary arteries of the type described in other vascular regions. However, this is mere speculation, since the results can be explained by the atherosclerotic changes in the coronary arteries.

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CORONARY CIRCULATION DURING PACING-INDUCED TACHYCARDIA

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Abstract. Coronary and systemic circulation has been studied in 10 patients with complete atrioventricular block and in 4 patients with sinus rhythm. Studies were performed during spontaneous rhythm, during pacing-induced tachycardia (PIT) of varying frequency and during a combination of PIT and exercise. Coronary flow and myocardial oxygen uptake varied linearly with heart rate. If exercise was superimposed on PIT with unchanged pacing frequency both coronary flow and myocardial oxygen uptake increased further. The coronary flow increased mainly because of a decrease in coronary vascular resistance. The results were compared with data from a previous study in which the heart rate was increased with exercise in a group of 11 patients without signs of major heart disease. In the PIT study the increase in coronary flow and myocardial oxygen uptake for a given increment in heart rate was less than half of that found in the previous exercise study. The main explanation for this difference is probably on the one hand that wall tension decreased during PIT while it increased during exercise and on the other hand that increase in contractility was more pronounced during exercise. The same relation of coronary flow or myocardial oxygen uptake to the product of systolic pressure and heart rate (BP × HR) was found in both groups although the values in the PIT study were in a lower range than those in the previous exercise study. This indicates that this product is a fairly good approximation of myocardial oxygen demand over a wide range of heart rates regardless of the method used for cardio-acceleration. *It is concluded from this study that PIT is less efficient than exercise in testing the coronary vascular reserve because the stress imposed on the myocardium is low during PIT even during high heart rates.*

Coronary circulation normally responds to increased myocardial oxygen demand with an increase of coronary flow. Different methods have been used in experimental animals and in man to increase oxygen demand of the myocardium, for example exercise (2, 6, 12, 14), isoprenaline infusion or other pharmacological agents (2, 26), and pacing-induced tachycardia (PIT) (3, 4, 7, 8, 9, 16, 20, 23, 24).

In coronary heart disease PIT has attracted increasing interest because of several specific advantages. The procedure is technically simple, the imposed stress is easy to grade and to reproduce and the ischemic symptoms are easily controlled by stopping the stimulation. There are also comparatively minor peripheral circulatory changes.

It has, however, not been established that the myocardial oxygen demand increases with increasing heart rate in the same fashion during PIT as during tachycardia caused by exercise. The purpose of this investigation was to study the effect of PIT on the coronary circulation and to compare the results with our previously published results on coronary circulatory changes during exercise (12).

METHODS

The study comprised 14 patients, 5 females and 9 males with a mean age of 59 years and a range from 30 to 72 years. Ten patients had a third degree atrioventricular block, 2 had sinus rhythm at the moment of the investigation but had had Adams Stokes attacks and had probably third degree block intermittently. Two patients had normal sinus rhythm and were investigated because of chest pain suspected to be anginal. The pertinent anthropometrical and clinical data are presented in Table 1.

The patients were investigated in the fasting state without premedication and in supine position. They were informed about the aim of the investigation and the procedure of the study and had given their consent. The catheterization technique, the equipment used and the method for determination of coronary flow has been described previously (11). A bipolar electrode catheter (USCI 5651) was also inserted in the right basilic vein. In those patients who had AV-block the tip was placed in the right ventricle, in those with normal AV conduction it was placed in the right atrium. An Elema pacemaker (Type 138) with variable frequency and amplitude was employed for pacing.

Coronary vascular resistance was calculated by dividing the mean arterial pressure by coronary flow and was expressed in mmHg/ml/100 g min. Coronary vascular

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Table I *Anthropometrical and clinical data*

Function group classification according to the N.Y. Heart Ass. Coronary arteriography findings classified from 1 to 5 (1 = no demonstrable vessel change, 2 = irregular vessel walls where no conspicuous decrease in vessel calibre could be seen, 3 = stenosis without delayed filling where the vessel lumen was narrowed by about one half, 4 = stenosis with delayed filling —filling of the vessel segments distal to the narrowing was delayed, 5 = occlusion, – = coronary arteriography not performed)

Pat no.	Clinical diagnosis	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m²)	Heart volume (ml)	Heart volume (ml/m² BSA)	Resting BP (mmHg)	Effort angina	Previous infarction	Funct. group	Cor. angio
1	AV-block III	♂	56	180	68	1.85	930	490	160/75	–	–	II	2
2	AV-block III + hypertension	♂	58	173	69	1.81	1 510	820	250/90	–	+	III	—
3	Sinus rhythm + Adams Stokes attacks	♂	69	165	69	1.75	—	450	150/80	–	–	I	—
4	Sinus rhythm + Adams Stokes attacks	♀	72	164	61	1.65	990	600	170/95	+	–	II	—
5	AV-block III	♀	45	165	62	1.66	690	430	145/75	+	–	II*	—
6	AV-block III + hypertension	♀	66	156	61	1.60	960	610	220/90	–	+?	III	—
7	AV-block III + aortic insufficiency	♂	54	175	73	1.86	1 040	560	140/70	–	–	II	1
8	AV-block III	♀	54	159	56	1.56	Enlargement		180/90	–	–	II	—
9	AV-block III + hypertension	♂	67	171	69	1.80	1 080	600	215/100	–	–	I	—
10	AV-block III	♂	72	177	76	1.93	1 480	770	200/85	–	–	I	—
11	AV-block II	♀	73	162	90	1.95	970	510	210/100	–	–	II	—
12	AV-block III	♂	68	176	63	1.76	740	410	230/95	–	–	II	—
13	Angina pectoris	♂	43	179	73	1.90	880	460	145/85	+	–	II*	—
14	Chest pain + St. post perimyocarditis	♂	30	176	80	1.96	700	360	120/80	–	–	I	I

* Patients in whom the limiting factor during daily life activities is angina pectoris. (In the other patients dyspnoea or fatigue in the legs is the limiting factor.)

conductance was calculated as the inverted value of coronary vascular resistance. Right atrial pressure was not subtracted from the arterial pressure since it was uniformly low. Oxygen saturation in arterial and coronary sinus blood and oxygen capacity were measured with methods described previously (5).

Procedure

The patients were investigated during different pacing-induced heart rates. In some of the patients the first study was done during slow idioventricular rhythm and in 7 of them the last studies were done on the same high heart rate, first at rest and then during exercise.

RESULTS

The results of the present study are given in Table II. These results are compared with the results from a control group of 11 subjects without signs of major heart disease where the heart rate was increased by exercise. They were studied with the same methods. A detailed description of the procedure and the results has been published elsewhere (12). The results from the control group are presented in the figures as regression lines for the group and in the text some values pertinent for the comparison are given.

Systemic Circulation

Increase in heart rate during PIT generally resulted in a fall of systolic pressure while diastolic pressure rose. The net result on mean pressure was a slight elevation. Within this general pattern there were wide individual variations. The cardiac

output increased up to heart rates approximately around 100 beats/min, and decreased again with higher heart rates. Stroke volume diminished suc cessively with increasing heart rate.

In the 7 patients, who performed exercise dur ing PIT all pressures rose to values above the resting and stroke volume and hence cardiac out put increased markedly

Coronary Circulation

Coronary flow in relation to heart rate

The coronary flow increased during pacing in a strikingly linear fashion over the whole range of heart rates. Similarly coronary flow increased with heart rate during exercise in the control subjects. However the increase in coronary flow for a given increase in heart rate was markedly smaller in the pacemaker group as compared to the control group. For comparison measurements in the control group were pooled and the regression line with 95% confidence limits was calculated, assuming that there was no individual effect in this group (Fig. 1 *a*).

The regression equations for the individual patients were also calculated in both groups. The mean value for the regression coefficient was in the present study 0.75 with a S.D of 0.29 and in the control subjects 1.57 with a S.D of 0.46. From the regression coefficients it could be calculated that for an increase in heart rate of 50 beats/min the coronary flow increased on an average 37 ml/100 g × min in the paced group while the same increase in heart rate resulted in a coronary flow increase of 78 ml/100 g × min in the control subjects.

Results from the 7 patients in whom coronary flow was measured on the same pacing-induced high heart rate first at rest and then during exer cise are presented in Fig. 1 *b* With a constant heart rate the coronary flow increased further with exercise.

Coronary flow in relation to the BP × HR product

The relationship between coronary flow and the product of systolic pressure and heart rate was approximately linear for each individual in both groups. The increase in coronary flow for a given increase of this product was of the same magnitude in both groups (Fig. 2). This was also reflected in the regression coefficient values for the individual patients. The mean value for the regression coefficient in the present study was 0.72 with a S.D of 0.38. In the control subjects the mean value was 0.76 with a S.D of 0.21 The values from the combined test of PIT and exercise in 7 patients in the present study had the same relationship between coronary flow and BP × HR product as the other values measured during PIT and there was no obvious difference as compared to the control group (Fig. 2 *b*)

Coronary vascular resistance in relation to BP × HR product

The coronary vascular resistance decreases continuously with increasing myocardial oxygen de mand and in the same fashion during pacing as in the control group. This was also true for the combination of PIT and exercise (Fig. 3). The values from the control group illustrate that the coronary vascular resistance decreases in a curvilinear fashion with increase in myocardial oxygen demand as in other vascular beds. To compare the two groups the coronary vascular conductance, the inverted value of coronary vascular resistance, was calculated. A linear regression analysis of the pooled data of the control group was performed. These values were compared to those obtained during pacing in the present study (Fig. 4) For any given value of the BP × HR product there was a tendency for the pacemaker group to have a lower coronary vascular conductance. The increase in coronary vascular conductance for a given increase in BP × HR product seemed to be the same in both groups.

Myocardial arteriovenous difference of oxygen in relation to BP × HR product

There was a fairly wide variation of a–v O_2 dif ference values in both groups but there was no systematic change with increasing BP × HR product within any of the groups. Both groups showed the same principal pattern. The only remarkable finding was that during the combination of PIT with exercise in the present study the a–v O_2 difference increased to unexpectedly high values in 5 of the 7 patients (Fig. 5).

Myocardial oxygen uptake in relation to heart rate

In both groups the myocardial oxygen uptake increased strikingly linearly with increasing heart

Table II *Hemodynamic and laboratory data*

R = rest, W = work, Sp = spontaneous heart rhythm (sinus or idioventricular), *HR* = heart rate (HR I = mean value during the investigation procedure, HR II = value recorded simultaneously with coronary flow measurement), CVR = coronary vascular resistance, CVC = coronary vascular condition

Pat. no.	Experimental condition		Work load (kpm/min)	HR I (beats/min)	HR II (beats/min)	Cardiac output (l/min)	Stroke volume (ml)	BP (mmHg) Aorta or brachial artery Syst.	Diast.	Mean	LA Mean	CS Mean
1	R	Sp	—	42	41	4.8	105	148	61	82	11	3
	R	PIT	—	43	70	3.8	80	143	74	89	8	3
	R	PIT	—	98	98	6.0	61	140	78	94	7	3
	W	PIT	200	98	98	7.6	78	170	71	102	14	5
2	R	Sp	—	56	36	8.9	159	148	51	79	5	3
	R	PIT	—	84	84	10.5	122	126	55	77	3	3
3	R	Sp	—	72	72	5.9	90	200	84	114	2	−2
	R	PIT	—	84	84	5.3	64	172	84	118	6	0
	R	PIT	—	99	98	5.3	53	156	83	114	3	0
4	R	Sp	—	62	62	3.0	51	149	78	90	7	3
	R	PIT	—	98	98	4.0	41	143	80	108	6	4
	R	PIT	—	148	148	3.4	23	130	88	108	12	5
	W	PIT	100	148	148	5.2	35	149	85	119	10	6
5	R	Sp	—	38	38	4.1	107	158	69	94	8	3
	R	PIT	—	84	84	4.5	53	132	87	99	4	2
	R	PIT	—	148	148	4.3	29	107	74	84	5	7
6	R	Sp	—	24	24	3.2	135	271	75	112	12	4
	R	PIT	—	85	85	4.3	51	176	104	130	6	0
	R	PIT	—	100	100	4.3	43	196	109	143	4	0
	R	Sp	—	63	60	3.2	80	137	59	86	13	3
	R	PIT	—	100	100	5.6	56	124	65	92	9	5
	W	PIT	200	100	100	7.6	76	162	65	97	15	9
8	R	Sp	—	43	43	2.9	64	148	52	90	13	5
	R	PIT	—	85	85	4.2	49	138	73	109	9	1
	R	PIT	—	150	150	2.9	19	137	85	106	20	7
	W	PIT	50	150	150	4.8	32	164	84	114	15	11
9	R	PIT	—	99	98	5.2	53	172	92	123	9	3
	W	PIT	200	100	100	9.1	91	183	83	137	16	7
10	R	PIT	—	100	100	5.0	50	174	89	123	5	3
	W	PIT	0	100	100	7.5	75	200	99	141	10	5
11	R	Sp	—	40	40	4.5	113	205	75	122	7	3
	R	PIT	—	70	71	5.6	80	150	80	107	5	3
	R	PIT	—	100	100	4.5	45	165	99	130	8	3
12	R	PIT	—	100	100	4.2	42	134	80	122	6	4
	W	PIT	100	100	100	6.8	68	159	88	117	9	8
13	R	Sp	—	67	70	6.8	101	164	85	116	11	2
	R	PIT	—	93	92	8.2	87	147	80	123	9	2
	R	PIT	—	120	120	9.3	78	138	86	109	6	−1
	R	PIT	—	150	150	8.7	57	134	95	112	7	−1
14	R	Sp	—	88	90	7.1	81	107	78	96	5	4
	R	PIT	—	100	100	7.8	78	116	82	93	4	4
	R	PIT	—	150	150	4.9	46	112	67	93	10	6

rate. An expression of this linear relationship is the correlation coefficient for the values of the individual patients. The mean value of correlation coefficients in the present study was 0.97 and in the control group 0.99.

There was a striking difference between the

Systolic pressure × heart rate 1/100	Arterial oxygen saturation (%)	Oxygen capacity (ml/l)	Coronary sinus oxygen saturation (%)	Myocardial arterio-venous oxygen difference (ml/l)	Coronary flow (ml/100 g min)	Myocardial oxygen uptake (ml/100 g min)	CVR (mmHg min 100 g ml)	CVC (1/CVR)
62	97.6	188	40.0	108	53	5.7	1.55	0.65
106	95.6	181	34.9	110	59	6.5	1.51	0.66
137	96.8	183	37.2	109	84	9.1	1.12	0.89
167	95.3	190	35.6	114	114	[illegible]	0.89	1.1
83	96.8	167	27.9	115	86	9.9	0.92	1.09
[illegible]	92.2	159	31.0	98	[illegible]	[illegible]	0.62	1.61
44	97.3	171	42.6	93	84	7.8	1.35	0.74
45	96.1	179	37.4	105	89	9.3	1.32	[illegible]
154	97.0	179	39.2	103	95	9.8	1.20	0.83
92	97.6	166	29.1	114	53	6.0	1.70	0.59
140	96.1	165	25.7	116	84	9.7	1.29	0.78
93	97.8	168	20.8	129	119	15.4	0.91	1.10
[illegible]	94.9	174	18.8	132	143	18.9	0.83	1.20
60	100	182	45	100	57	5.7	1.65	0.61
[illegible]	98.2	176	42.4	98	89	8.8	1.11	0.91
58	96.6	181	37.6	107	158	16.9	0.54	1.85
65	99.3	200	35.1	128	66	8.4	1.70	0.59
150	97.9	197	41.2	112	119	13.3	1.10	0.91
96	98.1	196	34.3	[illegible]	136	17.0	1.06	0.94
86	96.0	174	29.2	116	65	7.5	1.32	[illegible]
4	92.5	174	4.4	118	86	10.1	1.07	0.93
62	96.6	188	19.4	145	110	16.0	0.88	1.14
67	94.2	167	25.4	115	56	6.4	1.61	0.62
17	91.3	163	27.6	104	95	9.9	1.15	0.87
	93.8	168	33.1	101	129	13.1	0.82	1.22
46	93.5	173	27.4	115	143	16.4	0.80	[illegible]
170	97.7	208	77.0	146	86	12.6	1.43	0.70
83	92.7	209	21.9	148	102	15.1	1.15	0.87
174	98.9	190	25.8	139	98	13.6	[illegible]	0.80
200	99.1	198	25.7	145	130	18.6	1.08	0.93
82	97.3	171	25.1	123	63	7.7	1.93	0.52
105	95.6	173	[illegible]	123	82	10.1	1.31	0.76
165	97.8	171	28.4	119	102	12.1	1.27	0.79
134	97.9	186	29.6	127	110	14.0	1.10	0.91
159	97.4	188	23.8	138	136	18.8	0.87	1.15
110	97.0	175	31.7	114	73	8.3	1.58	0.63
137	95.1	175	32.4	110	95	10.5	1.29	0.78
166	94.6	168	36.3	98	119	11.7	0.92	1.09
[illegible]	99.3	168	36.3	106	158	16.7	0.71	1.41
94	97.6	203	38.3	123	66	8.0	1.45	0.69
116	98.3	205	40.3	119	68	8.1	1.37	0.73
168	98.7	198	32.1	132	89	11.7	1.04	0.96

groups in the rate of increase of myocardial oxygen uptake with increasing heart rate. The individual regression equations were calculated. The mean value of the regression coefficients in the present study was 0.81 with a S.D. of 0.19 and in the control group [illegible].35 with a S.D. of 1.35

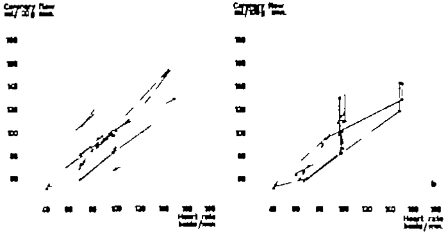


Fig. 1 a and b Relationship between coronary flow and heart rate, —— patients included in the present study ▲ measurements during spontaneous heart rhythm, sinus or idioventricular; ● measurements during PIT; ■, measurements during combination of PIT and exercise ---, the regression line and the 95% confidence limits of the pooled data from the control group.

Calculated from the individual regression equations there was in the present study an average increase of myocardial oxygen uptake of 4 1 ml/100 g × min for a pacing-induced increase of heart rate of 50 beats/min while in the control subjects the same increase in heart rate during exercise gave an increase of 9 8 ml/100 g × min (Fig. 6 *a*). In the combined test of PIT and exercise in 7 patients the myocardial oxygen uptake increased considerably compared to the value at rest with the same heart rate (Fig. 6 *b*). In the two patients in whom the test was performed with a heart rate of 150 beats/min the work load was much lower in relation to the heart rate than in control subjects. This probably explains why the measured ocardial oxygen uptake was still lower than was in the control subjects with the same heart ate

An interesting observation is that during extremely low heart rates in patients with third degree AV-block and idioventricular rhythm the myocardial oxygen uptake was not much lower than during the normal range of heart rates at rest.

Myocardial oxygen uptake in relation to BP × HR product

The myocardial oxygen uptake increased approximately linearly with increase in the BP × HR product in both groups. An expression of the linear relationship was the correlation coefficient of the regression equations of the individual patients. The mean value of the correlation coefficient in the present study was 0.92 and in the control subjects 0.98.

For any given value of the BP × HR product there was a tendency for the pacemaker group to have a lower myocardial oxygen uptake. The increase in myocardial oxygen uptake for a given increase in *BP × HR* product was approximately the same in both groups. The mean value of regression coefficients in the pacemaker group was

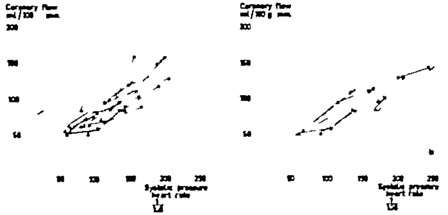


Fig. 2 and b Relationship between coronary flow and BP HR product. Symbols as in Fig. 1.

0.77 with a S.D. of 0.31 and in the control group 0.94 with a S.D. of 0.34 (Fig. 7 *a*). Values obtained during the combination of PIT and exercise showed the same type of relationship (Fig. 7 *b*).

DISCUSSION

The myocardial oxygen consumption is determined by several factors. The major determinants are heart rate, wall tension of the ventricle and state of contractility (25). PIT is of specific interest because it could induce gross changes in one factor heart rate, with only minor changes of the others. The wall tension could be expected to diminish somewhat because of the combination of slightly reduced systolic pressure and decreasing ventricular dimensions during PIT while the contractility increases moderately (1). The net effect of PIT on myocardial oxygen consumption could therefore be expected to be a continuous increase with increasing heart rate.

The principal response of the coronary circulation to increase of myocardial oxygen demand is an increase of coronary flow. The reports on the influence of PIT on coronary circulation are conflicting. In experimental animals in which flow has been studied with very accurate methods coronary flow increased continuously with increasing heart rate (4, 23). In man the same results have been reported in some investigations (8, 24). There are studies, however, showing a decrease in coronary flow on higher heart rates (3, 16). The discrepancies might to some extent be explained by different composition of patient materials and by different methods employed. Rubidium-84 used in one study (16), could have a slow diffusion as has been shown for rubidium-86 (19). This would result in diminishing extraction fraction of rubidium-84 in the myocardium with increasing coronary flow and this in turn would give erroneous flow values during high coronary flow. Difficulties in interpreting myocardial clearance curves during PIT have also been reported and might explain some of the diverging results (10).

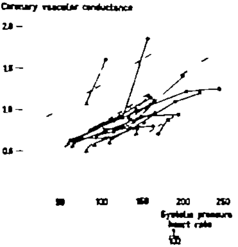


Fig. 4. Relationship between coronary vascular conductance and BP · HR product. Symbols as in Fig. 1.

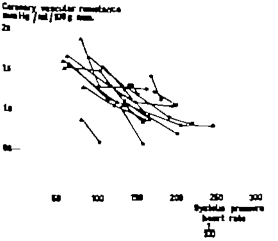


Fig. 3. Relationship between coronary vascular resistance and BP · HR product. Symbols as in Fig. 1.

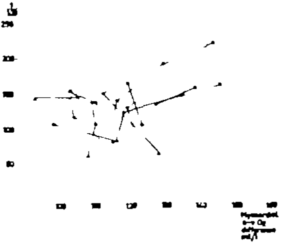


Fig. 5. Relationship between BP · HR product and myocardial arteriovenous oxygen difference. Symbols as in Fig. 1.

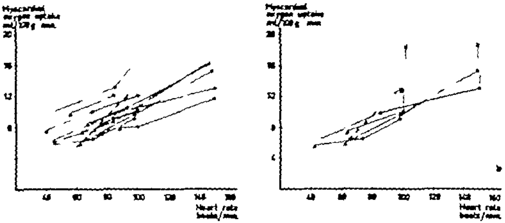


Fig. 6 a and b. Relationship between myocardial oxygen uptake and heart rate. Symbols as in Fig. 1

In the present study coronary flow increased linearly over the whole range of heart rates. This increase was mainly achieved through a decrease in coronary vascular resistance. The slight increase in driving pressure contributed relatively little. In the absence of major changes of sympathetic tone this change in coronary vascular resistance illustrates the accurate local metabolic regulation of blood flow. This accurate regulation was further demonstrated by the largely unchanged myocardial arterio-venous oxygen difference.

As expected the PIT resulted in an increase in myocardial oxygen uptake in the present study. The increase was linearly related to the heart rate increase. This supports the view that during PIT with varying heart rates the influence of other determinants of myocardial oxygen consumption is either small or of the same relative importance over the whole range of heart rates.

With PIT coronary flow, myocardial oxygen uptake and coronary vascular resistance changed in the same direction as during tachycardia induced by exercise but the changes were markedly less pronounced during PIT. Considering the major determinants of oxygen consumption it is easy to explain this different pattern of reaction. In PIT heart rate was the only oxygen determinant that changed markedly. In exercise other important changes paralleled the increase in heart rate. As a rule there was an increase of systolic pressure. The reports in the literature on cardiac dimensions during exercise are contradictory but the end-diastolic volume presumably changes little with heavy exercise (1, 13). The net effect in our study should then be an increase in myocardial wall tension. With moderate or heavy exercise there is also an increase in sympathetic tone resulting in a substantially greater improvement of

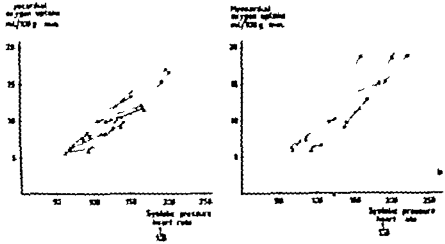


Fig. 7 a and b. Relationship between myocardial oxygen uptake and BP × HR product. Symbols as in Fig. 1.

the myocardial contractile state than during PIT (1 20). The parallel changes of these other factors during exercise explain the higher myocardial oxygen consumption for a given heart rate in exercise.

The results of these exercise and PIT studies demonstrate that heart rate alone is a poor measure of the total myocardial oxygen demand. Wall tension and contractility are not taken into account. These variables are, however still not accessible for direct measurement during this type of study Therefore, to the extent that systolic pressure in some way reflects these two factors, the product of heart rate and systolic pressure would be a better index of myocardial oxygen demand than heart rate alone. The present study conclusively shows that under the present conditions myocardial oxygen uptake can be reasonably well assessed by the BP × HR product irrespective of the method used to increase myocardial oxygen consumption. When this product is used the same type of relationship is found both during exercise, PIT and in combined trials of PIT and low levels of exercise.

It has been advocated that PIT should be used for testing patients with coronary heart disease. The method has, as already has been pointed out, technical advantages, and discomfort of the patient can be avoided by stopping the test. The present study however illustrates important shortcomings of the method. It is obvious that exercise puts a heavier load on the myocardium than PIT and that exercise therefore is a better test of the coronary reserve. For a given increase in heart rate the myocardial oxygen uptake increases more than twice during exercise compared to the increase during PIT Assuming a resting heart rate of 70 beats/min the results of this study indicate that PIT to 150 beats/min gives myocardial oxygen uptake values corresponding to what is achieved during exercise at a heart rate of 100 beats/min in the normotensive individual. A heart rate increase of this order during exercise tests corresponds to such a low work load that it will give only limited information about the coronary reserve in the majority of coronary patients.

The low level of myocardial stress imposed by PIT even during high heart rates explains why PIT as a means of inducing angina pectoris has been reported to give such varying results (3 9 14 17 20, 22).

The results of the present study could also be applied to other types of heart disease. PIT can be assumed to evoke hemodynamic changes similar to what is found in different types of supraventricular and ventricular tachycardias. This study thus suggests that all types of tachycardias without major concomitant increase in sympathetic tone result in only moderate increase in myocardial oxygen demand.

The results of the present study also emphasize that a correct interpretation of coronary flow changes is impossible without relating the coronary flow to some index reflecting the largest possible fraction of total myocardial oxygen demand. In our hands the BP × HR product has turned out to give a good approximation of the myocardial oxygen demand during different types of stimulation of the myocardium.

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ACKNOWLEDGEMENT

[illegible] [illegible] [illegible] by grants from the Swedish [illegible] [illegible] Council (B70-14X-100-05).

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ARRHYTHMIC COMPLICATIONS AFTER PARASYMPATHOLYTIC TREATMENT OF BRADYARRHYTHMIAS IN A CORONARY CARE UNIT

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Abstract. Parasympatholytic treatment of bradyarrhythmias is part of the policy in coronary care units (CCU). Because of psychotic central nervous reactions following repeated i.v. doses of atropine, this drug was replaced by methyl scopolamine, and no such reactions have occurred since then. During a period of continuous ECG-recording in a CCU 77 patients with bradyarrhythmias were given at least one dose of 0.12–0.5 mg methyl scopolamine. Its maximal effect as measured by the peak QRS-rate occurred after about 10 min. Within this time atrial fibrillation appeared for the first time in 2 patients, and ventricular tachycardia and ventricular fibrillation in another subject. During the following 2 hours no further major arrhythmias were observed. All of these arrhythmias occurred in the 16 patients with AMI and none in the 11 subjects with other diagnoses. The possibility of a causal relationship between treatment with methyl scopolamine and appearance of tachyarrhythmias is discussed. The arrhythmic complications described in this study have made us reduce the initial dose of methyl scopolamine from 0.5 to 0.06–0.12 mg, which often is satisfactory.

Bradyarrhythmias are a common feature early in acute myocardial infarction (AMI). Adgey et al. (1) found bradyarrhythmias in 61% of cases with acute posterior myocardial infarction in which monitoring was started within one hour from onset of symptoms. They recommend the use of atropine in small aliquots, preferably under monitoring, as ventricular ectopic rhythms may be precipitated.

Four kinds of arrhythmic complications have been reported following atropine administration. A nodal acceleration with A-V dissociation has been said to be common. It starts about 1 min after intravenous administration and lasts for about 2–3 min (7). Furthermore a few patients have been reported to develop supraventricular and ventricular ectopic beats after the administration of atropine (1, 4). Occasionally in patients with second degree A-V block the ventricular rates decrease although the atrial rates increase (1). We have found no report of ventricular tachycardia or fibrillation, except during anesthesia (3), nor of ectopic atrial tachyarrhythmias, following parasympatholytic treatment.

When using atropine in a dose of 0.5 mg intravenously in our CCU arrhythmic complications were observed within a few minutes in two patients. One patient developed ventricular fibrillation (see case report) and the other, who had a second degree A-V block, showed a decrease in ventricular rate. These two observations initiated the present study. In the meantime we had, however, replaced atropine by methyl scopolamine in an attempt to reduce the incidence of psychic reactions, i.e. confusion, paranoia and hallucinations (4, 5 and case report). Methyl scopolamine lacks the central nervous effects known to occur with atropine. As regards methyl scopolamine, no reports have been found about arrhythmic complications.

CASE REPORT

A 64-year-old woman was admitted to the CCU because of chest pain, with a history of left heart failure. ECG showed atrial fibrillation with a ventricular rate of 60–70/min. No signs of AMI appeared in subsequent laboratory investigations.

After 15 days of treatment with digoxin 0.25 mg, furosemide 80 mg and potassium chloride 3–4 mg daily digoxin was substituted for quinidine durules 1.2 g during one day and uncomplicated electrical cardioversion was performed on the following day. Atrial fibrillation was converted to nodal rhythm, 60 beats/min, with occasional supraventricular extrasystoles.

Eight hours later circulatory arrest occurred in the

Table I. *Indications for intravenous methyl scopolamine*

	No. of pats.
Sinus bradycardia (<50/min)	16
Sinus arrest	1
Second degree A-V block	5
Ventricular premature beats or ventricular escape rhythm in association with sinus rhythm ≤70/min	5

general ward, and following spontaneous recovery the patient was referred to the CCU with no signs of cerebral damage or heart failure. Digitalis was withheld. Because of episodes of nodal bradycardia repeated injections of 0.5 mg atropine were given, altogether 3.0 mg in 6 hours. This was stopped as no convincing effect on the slow rate was observed. Furthermore, at this time the patient had developed confusion and agitation which were interpreted as due to atropine. Two hours later an episode of ventricular tachycardia occurred, and an i.v. lignocaine infusion of 1 mg/min was started after an initial bolus of 50 mg. After 8 hours, during which time the psychic symptoms disappeared, although the nodal bradycardia persisted, 0.5 mg atropine was again tried. Within minutes the injection was followed by ventricular tachycardia and ventricular fibrillation. Prompt defibrillation was successful. The following day atrial tachycardia with a 2:1 block developed, making digitalis overdosage strongly suspected. One further episode of ventricular fibrillation another 12 hours later was not related to any atropine injection, and was successfully defibrillated. No more atropine was given; no further cerebral symptoms developed. Final recovery was essentially uneventful.

MATERIAL AND METHODS

In 202 consecutive admissions to the CCU during a 4-month period a continuous ECG was recorded by a multichannel ink-writing apparatus (Mingograph 81, Elema-Schonander, Solna, Sweden) with a paper speed of 10 mm/sec. The start of all injections was marked on the ECG. During this period 27 patients were given one or more doses of methyl scopolamine (Skopyl®) according to the indications in Table I.

Apart from these 27 patients another 4 were given methyl scopolamine but were not included in the study as the main indications were atrial fibrillation (with slow ventricular rate) or recurrent ventricular tachycardia (with slow basic rhythm). Of the 27 patients 16 were subsequently diagnosed as AMI (9 men and 7 women, aged 45–85 years, mean 64 years). Four of the infarcts were predominantly anterior and nine inferior. In three instances the diagnosis was based only on a typical enzyme pattern and history. The 16 patients were admitted 1–28 hours, mean 6 hours, after the onset of symptoms, and they were given the first dose of methyl scopolamine 0–63 hours, mean 8 hours, after the admission.

In the beginning of the study the first dose was 0.5 mg; but after ventricular fibrillation had been observed in one patient following this dose, it was reduced to 0.25 mg. Later it was further reduced to 0.12 mg because of subsequent atrial fibrillation in another case.

The ECGs of the 27 patients were analysed for the QRS-frequency and major arrhythmias from the admission until 2 hours after their first dose of methyl scopolamine. The incidence of supraventricular and ventricular premature beats/min was calculated during 10 min before and after the administration of methyl scopolamine.

RESULTS

The effect of 0.25 mg of methyl scopolamine on the QRS-rate is presented in Fig. 1. The QRS-rate rose rapidly during 3 min following the injection, with near maximal values after 3 min, and the peak seems to be reached at about 10 min.

Among the 5 patients with second degree A-V block none showed a significant decrease in QRS-rate, which may be compared with 2 out of 11 patients reported by Adgey et al. (1).

The patients with significantly increased frequency of premature beats or first appearance of major arrhythmias are presented in Table II.

The development of nodal rhythm was thus rather common, as has been reported previously (2) and it was predominantly seen in patients without a proven AMI.

Regarding premature beats there was a highly significant increase of supraventricular ectopics in one patient, and of ventricular premature beats in two others. In all three patients the increase appeared within 2–5 min. The two patients with an increased incidence of ventricular premature beats had an AMI and in one of them the ectopics were often of R-on-T type and occasionally paired.

In two other patients with AMI, atrial fibrillation was recorded for the first time during their

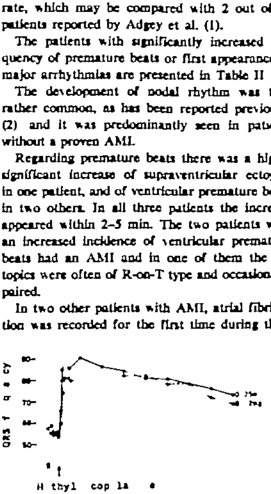


Fig. 1. QRS-rate following mg methyl scopolamine injection.

stay 5 min following the injection of methyl scopolamine. In one of them, a 77-year-old man, the atrial fibrillation gave rise to QRS-rates of about 150/min and was followed by pulmonary oedema within a few minutes. DC conversion restored sinus rhythm, but atrial fibrillation soon recurred. Despite intensive treatment the patient died in frank pulmonary oedema about 2 hours after the injection of methyl scopolamine. Post mortem examination showed very extensive myocardial infarctions with engagement of about 85% of the left ventricle.

Another patient with acute anterior myocardial infarction was on prophylactic lignocaine infusion, 2 mg/min, when during vomiting sinus bradycardia occurred, followed by sinus arrest during 16 sec. Methyl scopolamine 0.5 mg was given, and the lignocaine infusion was stopped. Ventricular tachycardia appeared for the first time 5 min after the injection of methyl scopolamine, and was followed 4 min later by ventricular fibrillation, which was successfully treated.

All of these major arrhythmias occurred within the first 9 min following the methyl scopolamine injection, and during the remaining 120 min analysed no further onset of major arrhythmias was found.

Table II. *Arrhythmias following intravenous methyl scopolamine*

NR = nodal rhythm, SVPB = supraventricular premature beats, VPB = ventricular premature beats, AF = atrial fibrillation, VT = ventricular tachycardia, VF = ventricular fibrillation

AMI/site	Developing arrhythmias	Time after injection (min)	Injected dose (mg)
None	NR	2 3	0.25
None	NR	3–5	0 12
None	NR	4–5	0.12
None	NR	2–	0.12
None	NR	3–4	0.12
	SVPB	5–	
Anterior	NR	1	0.50
	VPB	2	
Undetermined	VPB	3–	0 12
Posterior	AF	5–	0.25
Posterior	AF	5–	0.12
Anterior	VT	5	0.50
	VF	9	

DISCUSSION

Besides the minor rhythm disturbances known to occur following atropine administration, major arrhythmias developed in 3 patients out of 27 treated with methyl scopolamine intravenously in the present series. This may be coincidental, but two facts speak in favour of a causal relationship. 1) These major arrhythmias appeared within 10 min, just before the peak action of methyl scopolamine as measured by the QRS-rate increase. No further major arrhythmias occurred during the subsequent 110 min. 2) The major arrhythmias seen in these three patients were atrial fibrillation, ventricular tachycardia and ventricular fibrillation. As known, these arrhythmias may be preceded by frequent supraventricular ectopic beats and ventricular premature beats, and these significantly increased in incidence within 5 min following methyl scopolamine in another three patients. These two circumstances seem to support a causal relationship between intravenous methyl scopolamine treatment and subsequent major arrhythmias.

All the 3 patients developing major arrhythmias were among the 16 with a proven AMI. None was seen among the 11 subjects with other diagnoses.

It has been recommended that the dosage of methyl scopolamine should be kept at one third of that used for atropine (4). The arrhythmias occurred both after 0.5 0.25 and 0.12 mg, and these doses of methyl scopolamine can therefore not be said to have been especially high. It seems impossible to conclude from the literature whether methyl scopolamine is more prone to cause arrhythmias than corresponding doses of atropine. However as previously mentioned, we have also observed the onset of ventricular fibrillation within a few minutes following 0.5 mg of atropine.

As regards psychic reactions, methyl scopolamine seems to be preferable to atropine. During one year's experience of methyl scopolamine we have not seen any patient showing psychic reactions, which is in contrast to several such episodes in patients following a few doses of 0.5 mg of atropine during the preceding 1 1/ years.

It would be valuable to be able to predict these adverse responses in heart rhythm. The 3 patients with major arrhythmias did not differ from the other 13 with an AMI as regards age, delay between onset of symptoms and admission to the unit, or the site of infarction. Nor did they dif

fer regarding the indication for methyl scopolamine or the time interval between admission and the first dose. There was no difference in treatment before methyl scopolamine, including previous digitalis therapy.

The figures in the present series are small but support an association between methyl scopolamine treatment and subsequent ectopic supraventricular and ventricular tachyarrhythmias in patients with acute myocardial infarction.

ACKNOWLEDGEMENT

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RESPIRATORY MECHANICS IN UNTREATED MYASTHENIA GRAVIS WITH SPECIAL REFERENCE TO THE RESPIRATORY FORCES

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Abstract. The respiratory mechanics have been studied in nine patients with myasthenia gravis in the untreated state. Comparisons have been made with the findings in healthy controls. Although the patients mainly had moderate myasthenia gravis the maximum respiratory pressures as expressions of the respiratory muscle strength were significantly decreased. Vital capacity, functional residual capacity, total lung capacity and the dynamic lung volumes were also diminished. No mean changes in lung resistance or lung compliance were shown to exist in the group. The relative mean value of maximum expiratory pressure (P_Emax) (the observed value in per cent of the predicted normal mean value) was lower than any other relative lung function variable. Thus the maximum inspiratory force (P_Imax), of paramount importance for the ventilatory capacity, was systematically less decreased than P_Emax. Even after consideration of the effect of decreased respiratory muscle strength vital capacity, total lung capacity and functional residual capacity were still diminished. It is suggested that decreased physical activity might diminish these static lung volumes via low thoracic compliance. The results also suggested another volume–pressure relationship between vital capacity and P_Imax, as is found in control cases with normal muscular strength. Otherwise similar functional relationships were at hand. The decreased values of dynamic lung volumes in comparison with those predicted only from anthropometric variables could only be explained to a minor extent by the direct influence of decreased respiratory muscle strength. The differences were mainly mediated via changes in the vital capacity which is dependent on the respiratory force variables.

The involvement of the respiratory muscles in myasthenia gravis is of great prognostic importance (19, 31). Weakness of the respiratory muscles (41) or even respiratory insufficiency (7) may occasionally be the first manifestations of myasthenia gravis although respiratory insufficiency is usually seen in connection with more general and serious manifestations of this disease (31, 41). The risk of respiratory insufficiency may be increased by infectious states (19, 31), in particular respiratory infections (7, 30, 40, 44), and after surgery (22, 26, 30, 44). When death occurs as a result of this disorder respiratory failure is a common denominator (16).

This indicates that information about the respiratory forces and their influence on other components of respiratory mechanics is of interest in myasthenia gravis. The present work deals with this problem as no earlier study seems to exist.

MATERIAL

Nine patients—five women and four men, aged 17 to 64—with moderate to rather severe myasthenia gravis were examined. The examinations were carried out during the years 1964 to 1969 in connection with the first hospitalization of these patients at the Department of Neurology, University Hospital, Umeå, except one patient (no. 1), who was examined at the Hospital of Vänersborg. The diagnosis was based on the course of the disease and the neurological findings. Patients with myasthenia gravis limited to ocular or bulbar muscles were not included in the investigation nor were patients with primary lung disease. A summary of clinical data at the time of examination is given in Table I. Table II gives the maximum voluntary isometric strength in the trunk, elbow and finger flexors examined in close conjunction with the lung function studies and at the same time of the day, namely in the morning.

During the examination period only patient 4 smoked. Patient 8 had stopped smoking two months before the examination. Previously he had been a heavy smoker (20–40 cigarettes a day).

The results presented are derived from the first complete examination of lung function before and after anticholinesterases and sympathomimetics (37). In patients 1–4, 7–9 this corresponded to the first complete examination while in the others partial lung function studies had been performed earlier with similar results.

The results obtained from the myasthenia gravis pa-

Table I. *Clinical data for the myasthenia gravis patients at the time of examination*

Pat. no.	1	2	3	4	5	6	7	8	9
Sex	♀	♀	♂	♀	♂	♂	♀	♂	♀
Age (y)	55	30	45	31	64	28	17	59	33
Height (cm)	165	171	185	158	184	165	162	180	183
Weight (kg)	68	70	83	50	92	58	48	86	51
Duration of disease (y)	31	6	2	1	2	4	2	1	3
Duration of anticholinesterase medication (y)	26	2	–	–	1	1	–	–	–
Thymectomy (age)	–	–	–	–	–	–	–	–	–
Respiratory distress	–	–	–	–	–	–	–	(+)	(+)
Facies myasthenica	–	+	–	+	+	+	+	(+)	+
Ptosis	+	+	–	+	–	+	+	+	+
Diplopia	–	+	–	+	+	–	–	+	+
Dysphagia	–	–	–	+	+	–	–	+	+

tients have been compared with the results from a healthy control series (35, 36, 38). For the present purpose it is desirable to have a healthy series for comparison, which exhibits the characteristics necessary to be representative for the healthy population, e.g. random sampling of subjects, comparable, valid measuring equipment and procedures, preferably carried out in the same laboratory. With one exception (non-random sampling) the control series fulfils these criteria for all practical purposes. Comparative studies have shown the effect of the non-random sampling to be small (15). The healthy control series is commented upon in greater detail elsewhere (35). Results from several other healthy series have been shown to be in good agreement with the findings in this control series (15).

METHODS

The isometric strength in trunk flexion, elbow flexion and handgrip was determined with dynamometers of essentially the same type as described by Asmussen et al. (4). The procedures have been described earlier (4, 35).

General procedure for the lung function studies. The examinations were carried out in the morning. The patients were either previously untreated or the medicine had been withdrawn at least 12 hours before the start of the examination. The patients were examined in sitting position. The measurements were made in the following order: static and dynamic lung volumes, maximum respiratory pressures, and about half an hour later lung compliance and lung resistance, all together comprising a time period of two to three hours with due allowance for rest between the different procedures.

A detailed description of the equipment and procedures used for the *determination of static and dynamic lung volumes and lung resistance* has been given elsewhere (35) and is in general agreement with that normally used (3, 6, 9, 11, 17, 21, 24, 28).

The maximum expiratory and inspiratory forces were measured as the highest positive and negative pressures which could be developed during an instantaneous and, for all practical purposes, isometric effort from the level of full inflation and expiration respectively (35). A leak from the mouth was used, as described earlier, to avoid the recording of a pressure created by the mouth. The pressure measurements were made with an inductive pressure transducer (Elema-Schönander EMT 490 A) with appurtenant amplifier and recorded within the linear range of Mingograph galvanometers.

Normal values (NV) were predicted from regression equations of absolute values obtained from analyses of the series studied by T. Ringqvist (35). The isometric strength of the elbow flexors, trunk flexors and handgrip was predicted from regression equations with sex, age (age – mean age)² and height as regressors. The normal limits have been defined as the mean value ±2 S.D. from the values from the controls. The regression equations for the dynamic lung volumes have been published earlier (35) and are to be published separately for the static lung volumes (34), lung resistance and compliance (36).

Relative values. To obtain an interindividual and intervariable standardization and to diminish possible skewness of the absolute values, these were converted to percentages of the predicted individual values (% NV), i.e. to "relative" values (Tables III and IV).

Table II. *Muscular strength in the myasthenia gravis patients*

NV = normal value

Pat. no.	Trunk flexion		Elbow flexion		Handgrip	
	kg	% NV	kg	% NV	kg	% NV
1	30	95	15	80	30	90
2	15	41	6	30	27	75
3	45	71	15	47	31	57
4	19	59	9	53	19	59
5	30.5	56	22.5	78	39.5	80
6	34	54	19	64	33	68
7	18	46	8	44	19.5	63
8	51.5	89	30.5	102	43	88
9	17	51	6	1	4.5	13
Mean	30.5	62.4	14.6	57.7	38.8	65.9

Table III *Observed absolute and relative values for lung resistance (Ra), compliance (Co), maximum respiratory pressure ($P_E max$, $P_I max$), and static lung volume in the myasthenia gravis patients and in the control series (mean values, medians and S D)*

VC = vital capacity FRC functional residual capacity RV = residual volume, TLC = total lung capacity The mean intra-individual differences (Diff.) between observed and predicted values are also given for the myasthenia gravis group

Pat. no.	Ra cm H_2O l/sec	% NV	Co l/cm H_2O	% NV	$P_{E}max$ cm H_2O	% NV	$P_{I}max$ cm H_2O	% NV	VC l BTPS	% NV	FRC l BTPS	% NV	RV l BTPS	% NV	TLC l BTPS	% NV
1	3.2	91	0.11	69	128	83	109	128	3.37	95	2.33	87	1.43	87	4.79	92
2	2.3	82	0.14	78	122	76	111	111	4.29	98	3.13	105	1.23	85	5.52	96
3	2.2	79	0.22	88	166	69	144	109	4.86	81	3.44	77	1.61	72	6.47	78
4	1.8	56	0.19	136	56	33	95	86	2.66	73	1.95	84	1.03	88	3.69	80
5	2.6	90	0.25	100	124	56	79	69	4.82	89	3.62	82	2.17	83	6.99	85
6	3.6	124	0.15	71	119	47	74	52	3.93	78	2.74	79	1.43	98	5.36	84
7	2.5	100	0.10	67	60	34	59	50	2.21	54	2.24	89	1.63	138	3.84	78
8	4.5	150	0.12	52	161	71	55	47	3.02	57	2.72	65	2.07	85	5.09	66
9	2.5	81	0.13	81	45	27	48	46	2.83	73	2.90	112	1.93	148	4.76	94
Mean	2.8	95	0.16	82	109	55	86	78	3.55	78	2.79	87	1.61	98	5.17	84
Median	2.5	90	0.14	78	122	56	79	69	3.37	78	2.74	84	1.61	87	5.09	84
Diff	−0.2	12	0.04	18	−88	45	28	−22	−1.03	−22	0.50	−13	−0.11	−2	−1.08	−16
Controls																
Females																
Mean	3.3				165		94		3.4		2.5		1.5		4.9	
S.D	1.1				29		24		0.7		0.5		0.4		0.8	
Males																
Mean	2.9				230		123		5.1		4.0		2.2		7.3	
S.D	1.0				47		30		1.0		0.9		0.6		1.2	

$p < 0.05$; ** $p < 0.01$ p 0.001

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Duration of anticholinesterase medication (y)	26	2	–	–	1	1	–	–	–
Thymectomy (age)	–	–	–	–	–	–	–	–	–
Respiratory distress	–	–	–	–	–	–	–	(+)	(+)
Facies myasthenica	–	+	–	+	+	+	+	(+)	+
Ptosis	–	+	–	+	–	+	+	+	+
Diplopia	–	+	–	+	+	–	–	+	+
Dysphagia	–	–	–	+	+	–	–	+	+

tients have been compared with the results from a healthy control series (35, 36, 38). For the present purpose it is desirable to have a healthy series for comparison, which exhibits the characteristics necessary to be representative for the healthy population, e.g. random sampling of subjects, comparable, valid measuring equipment and procedures, preferably carried out in the same laboratory. With one exception (non-random sampling) the control series fulfils these criteria for all practical purposes. Comparative studies have shown the effect of the non-random sampling to be small (15). The healthy control series is commented upon in greater detail elsewhere (35). Results from several other healthy series have been shown to be in good agreement with the findings in this control series (15).

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The maximum expiratory and inspiratory forces were measured as the highest positive and negative pressures which could be developed during an instantaneous and, for all practical purposes, isometric effort from the level of full inflation and expiration respectively (35). A leak from the mouth was used, as described earlier to avoid the recording of a pressure created by the mouth. The pressure measurements were made with an inductive pressure transducer (Elema-Schönander EMT 490 A) with appertenant amplifier and recorded within the linear range of Mingograph galvanometers.

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Relative values. To obtain an interindividual and intervariable standardization and to diminish a possible skewness of the absolute values, these were converted to percentages of the predicted individual values (= NV), i.e. to relative values (Tables III and IV).

Table II. *Muscular strength in the myasthenia gravis patients*

NV = normal value

Pat. no.	Trunk flexion kg	% NV	Elbow flexion kg	% NV	Handgrip kg	% NV
1	30	95	13	80	30	90
2	15	41	6	30	27	75
3	43	71	15	47	35	57
4	19	59	9	53	19	59
5	30.5	56	22.5	78	39.5	80
6	34	54	19	64	33	68
7	18	46	8	44	19.5	63
8	51.5	89	30.5	102	43	88
9	37	51	6	21	4.5	13
Mean	30.5	62.4	14.6	57.7	28.8	65.9

Table V *Differences between observed mean values (Obs.) and predicted values (Pred.)*

Pred. A, from anthropometric variables. Pred. B, the maximum respiratory pressure (P_{max}) also included (eq. 1–5) Pred. C, vital capacity and lung resistance also included (eq. 6–8). Regression equations according to T. Ringqvist (35) and T. Ringqvist & I. Ringqvist (34). Regressors with coefficients of the 10 % significance level are included. Abbreviations as in Tables III and IV

	Pred. A	Difference Obs. – Pred. A	Pred. B	Difference Obs. – Pred. B	Pred. C	Difference Obs. – Pred. C
VC	4.59	−1.03	4.51	−0.96	—	—
FRC	3.37	−0.50*	3.75	−0.96	—	—
RV	1.59	−0.11	1.98	0.36	—	—
TLC	6.26	−1.08	—	—	—	—
FEV	3.60	−0.68	—	—	2.90	0.02
FIV	3.88	−0.58	3.76	−0.43	3.00	0.31
MVV_{80}	144	−35	135	−26	120	−11

Eq. no.	Regressand	Coefficients and regressors
1	VC	-0.63 Sex $- 0.019$ A $+ 0.067$ H 0.0041 $P_I$max $- 5.51$
2	FRC	-1.03 Sex $+ 0.052$ H $- 0.0043$ $P_E$max $- 3.04$
3	RV	-0.63 Sex $+ 0.016$ A $+ 0.019$ H $- 0.0026$ $P_E$max $- 0.62$
4	FIV	-0.40 Sex $- 0.018$ A $+ 0.058$ H $+ 0.0085$ $P_I$max $- 5.49$
5	MVV_{80}	$-$ 4.8 Sex $- 1.141$ A $+ 1.1$ 4 H 0.095 ($P_I$max $P_E$max) $+ 0.647$ F $- 42$
6	FIV	-0.005 A $+ 0.0052$ $P_I$max 0.775 VC $- 0.01$
7	$FEV_{1.0}$	-0.016 A $+ 0.0019$ $P_E$max $+ 0.55$ VC $- 0.14$ Re 1.77
8	MVV_{80}	-14.4 Sex $- 0.775$ A $+ 0.1073$ ($P_I$max $- P_E$max) 13.23 VC $- 5.62$ Re 0.588 F 74.7

$p<0.05$ $p<0.01$ $p<0.001$

Deviation from normal values as predicted from anthropometric variables

Maximum voluntary isometric strength. The relative values of the maximum voluntary isometric strength of trunk and elbow flexors together with handgrip showed considerable interindividual variations with regard to each of these muscle groups (Table II). On average the muscle strength was decreased to about 55–65 % of the predicted normal mean values for these muscle groups ($p < 0.01$).

The $P_E$max were highly significantly lower ($p < 0.001$) for the myasthenia group than the predicted normal values, i.e. 100 % (Table III). The mean value of the relative $P_E$max, about 55 % was about the same as the relative values of the studied muscle groups. In five patients P max was below the normal lower limit (mean − 2 S.D.).

Relative $P_E$max for the group was lower than all the relative static and dynamic lung volumes. The difference being probably significant ($p < 0.05$) for the relative residual volume and total lung capacity and significant ($p < 0.01$) for vital capacity functional residual capacity FEV, FIV and MVV_{80}. (FEV = forced expiratory volume in one second—l BTPS or in % of NV $FIV_{1.0}$ = forced inspiratory volume in one second—l BTPS or in % of NV MVV_{80} = maximum voluntary ventilation performed at a metronome frequency of 80/min—l BTPS/mm or in % of NV.)

There were great variations in the group for the *maximum inspiratory pressure* ($P_I$max) with values ranging from over the predicted mean values to values below the lower normal limit (Table III). Relative $P_I$max was smaller than relative $P_E$max in one patient (no. 8) indicating a systematically smaller involvement of the inspiratory muscles ($p < 0.05$). On average the maximum inspiratory strength was not decreased to a greater extent than the maximum strength of trunk flexors, elbow flexors and handgrip There was a fairly good correlation between relative $P_E$max and P max ($r = 0.67$). Between relative $P_E$max and trunk flexion the correlation was not significant ($r = 0.58$).

Static lung volumes (Table III). The *total lung capacity* was in all nine patients lower than the predicted value ($p < 0.01$). The deviations were on the whole comparatively small, i.e. the average value corresponded to 83 % of the predicted mean value and only one subject (no. 8) was below the lower normal limit. For the whole group, and in this patient especially the major decrease was found in the *vital capacity* which was below the

Table VI. *Multiple regression analyses of the myasthenia patients*

Regression coefficients of the 10% significance level are given obtained from stepwise regression. Abbreviations as in Tables III and IV

Eq. no.	Regressand	Regressors	R.S.D.	R^2
1	VC	0.37 H 0.014 $P_I$max − 7.33 Not significant: Sex, $P_I$max	0.58	0.73
2	FRC	0.044 H − 4.70 Not significant. Sex, A, $P_E$max	0.33	0.67
3	RV	0.024 H − 2.53 Not significant. Sex, A, $P_E$max	0.34	0.38
4	TLC	0.089 H − 9.93 Not significant: Sex, (A 50)^2 $P_E$max, $P_I$max	0.65	0.69
5	FEV	0.021 H 0.974 VC + 0.29 Not significant: Sex, (A−50) $P_E$max, Re	0.22	0.97
6	$FIV_{1.0}$	0.904 VC 0.10 Not significant. Sex, A, (A 50)^2 $P_I$max, H, $P_E$max	0.33	0.89
7	MVV_{80}	−1.302 A 17.65 VC + 0.426 ($P_I$max + $P_E$max) + 15.31	13.8	0.92

$p < 0.05$ $p < 0.01$ $p < 0.001$

predicted mean value in all nine patients ($p < 0.01$). For the *residual volume* there was no certain discrepancy between predicted and observed mean values. The *functional residual capacity* for the group was found to be probably significantly lower ($p < 0.05$) than the predicted values.

Lung mechanics. There was no systematic difference for the group between observed *lung resistance* values and those predicted from anthropometric variables (Table III), which also applies to the *lung compliance* with respect to observed and predicted relative values. A probably significant difference ($p < 0.05$) was, however found between the observed and predicted absolute values, the former being smaller. After consideration was taken to the lung volumes and lung resistance (36) no significant difference was at hand between the observed and predicted absolute mean values of lung compliance.

Dynamic lung volumes. The observed *forced expiratory volume in one second* ($FEV_{1.0}$) was lower than predicted in eight out of nine cases while corresponding comparisons for *forced inspiratory volume in one second* ($FIV_{1.0}$) and *maximum voluntary ventilation* with breathing frequency 80/min (MVV_{80}) showed lower values than were predicted in seven patients (Table IV). These differences were probably significant ($p < 0.05$). A comparison between the first and the second half of MVV_{80} did not show any significant difference for the group as a whole.

Additional value of maximum respiratory pressures ($P_I$max and $P_E$max), vital capacity and lung resistance for predicting lung volumes. The discrepancies found when comparing observed values and those predicted from anthropometric variables may be due to changes in other variables caused by the disease. Comparisons between observed values in the myasthenia gravis patients and those predicted from regression equations obtained from the controls, also using variables of possible causal importance besides the anthropometric measures, have therefore been carried out (35–38) (Table V).

Considering the *static lung volumes* there was no change in the difference for the vital capacity while an increase was found for functional residual capacity and residual volume when Pmax was accounted for (Table V).

No significant influence on the total lung capacity of the maximum respiratory forces was found in the controls (38) and no further comparison could therefore be carried out for this variable.

The dynamic lung volumes were in this part of the analyses predicted first by the additional consideration of only the relevant maximum respiratory pressure (pred. B Table V). In the second place the vital capacity and the lung resistance in

Table VII. *Multiple regression analyses of the myasthenia patients*

The regression coefficients have been obtained from stepwise regression. The same regressors as for the control series are included. Abbreviations as in Tables III and IV

Eq. no.	Regressand	Regressors	R.S.D	R^2
1	VC	$0.47\ Sex - 0.0027\ A + 0.040\ H + 0.015\ P_I max - 4.65$	0.68	0.75
2	FRC	$0.11\ Sex + 0.061\ H - 0.0030\ P_E max - 7.16$	0.32	0.73
3	RV	$0.28\ Sex + 0.0115\ A + 0.028\ H - 0.0079\ P_E max - 2.93$	0.32	0.68
4	TLC	$0.16\ Sex + 0.13\ H - 17.3$	0.97	0.74
5	FEV	$-0.020\ A + 0.007\ P_E max + 0.752\ VC - 0.346\ Ra + 1.23$	0.35	0.93
6	FIV	$-0.011\ A + 0.0091\ P_I max + 0.843\ VC - 0.09$	0.37	0.91
7	MVV_{40}	$15.72\ Sex - 1.085\ A - 0.2743\ (P_I max + P_E max) - 28.66\ VC + 5.27\ Ra + 0.361\ F - 68.6$	16	0.95

In eq. 1–3 Sex = 0 for females and 1 for males.
In eq. 4–7 Sex = 2 for females and 1 for males.

$FEV_{1.0}$ and MVV_{40} were also considered (pred. C, Table V) according to regression equations from the controls (35). By this means the significant differences that still existed in the former situation between observed and predicted values could be eliminated in the latter when vital capacity and lung resistance were also considered. With regard to $FIV_{1.0}$ an overcompensation occurred so that the observed mean value became significantly higher than the predicted one.

Analyses of regression coefficients in the myasthenia series. The respiratory forces in the myasthenia gravis series were found to be of importance only for the vital capacity of the *static lung volumes* (Table VI, eq. 1–4). Multiple regression analysis thus indicated a decrease in this variable for smaller inspiratory forces.

For the *dynamic lung volumes* significant, positive effects on MVV_{40} of both the respiratory forces and the vital capacity simultaneously considered, were shown to exist. With respect to $FIV_{1.0}$ and FEV_1 no certain Pmax influence was found in the statistical analyses.

Comparisons of regression coefficients in the healthy and myasthenia series

The discrepancies found between observed values of lung volumes and those predicted, even when Pmax, vital capacity and lung resistance were considered, could in some degree be due to different functional relationships between the lung volumes and the regressors in the myasthenia and healthy series. To analyse this comparisons were made of regression coefficients from identical equations (Tables V and VII) for the various regressands (lung volumes) studied, using absolute values. For all static and dynamic lung volumes as regressands no difference between the coefficients for the causal regressors used could be found between the controls and the myasthenia series. The coefficient of $P_I max$ to predict vital capacity in the myasthenia gravis series was, however almost significantly higher ($0.05 < p < 0.01$) than in the control series with a value about four times higher in the myasthenia series than in the controls.

DISCUSSION

Weakness and rapid exhaustion of the striated muscles are characteristic symptoms of myasthenia gravis (31, 41, 43). The impact of these symptoms on the respiratory forces has not been analysed earlier nor is there any study concerning the relationship between decreased maximum respiratory muscle force and other variables of respiratory mechanics.

The present group of myasthenia gravis patients is small but comprises patients with varying degrees of decreased muscular strength. Attention has been primarily focused on obtaining information concerning respiratory muscle impairment, and the resulting effects on other variables of respiratory mechanics, and to some extent also comparisons with the involvement of some other skeletal muscle groups.

Table VI. *Multiple regression analyses of the myasthenia patients*

Regression coefficients of the 10% significance level are given obtained from stepwise regression. Abbreviations as in Tables III and IV

Eq no.	Regressand	Regressors	R.S.D	R^2
1	VC	0.57 H + 0.014 $P_I$max − 7.33 Not significant. Sex, $P_I$max	0.58	0.73
2	FRC	0.044 H − 4.70 Not significant. Sex, A, P max	0.33	0 67
3	RV	0 024 H − 2.53 Not significant Sex, A, $P_E$max	0.34	0.38
4	TLC	0.089 H − 9.93 Not significant: Sex, $(A-50)^2$ $P_E$max, $P_I$max	0.65	0.69
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7	MVV_{80}	− 1.302 A + 17.65 VC + 0.426 ($P_I$max + $P_E$max) + 15.31	13.8	0.92

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Lung mechanics. There was no systematic difference for the group between observed *lung resistance* values and those predicted from anthropometric variables (Table III) which also applies to the *lung compliance* with respect to observed and predicted relative values. A probably significant difference ($p < 0.05$) was, however found between the observed and predicted absolute values, the former being smaller After consideration was taken to the lung volumes and lung resistance (36), no significant difference was at hand between the observed and predicted absolute mean values of lung compliance.

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The *dynamic lung volumes* were in this part of the analyses predicted first by the additional consideration of only the relevant maximum respiratory pressure (pred. B, Table V). In the second place the vital capacity and the lung resistance in

not seem to have interfered with the resistive properties of the lung. An inadequate drainage of the airways is known to increase the susceptibility to recurring muco-purulent infections (13).

Dynamic lung volumes may be conceived of as dependent on variations in the displaceable lung volume available and/or in the forces that generate or oppose alternating movements of the *respiratory forces.* A *decrease in the ventilatory* capacity as found in the present series, was to be expected as the vital capacity and the maximum respiratory forces were decreased. The further analyses also indicated a good agreement between observed and predicted values for the dynamic lung volumes studied, when these causally related variables were accounted for in contrast to when the conventional anthropometric regressors were used. This implies among other things that the functional relationships found for healthy subjects (35) are to some extent also valid for subjects with decreased respiratory force. This is also suggested by the fact that no significant differences were found between corresponding regression coefficients in the control and myasthenia series with respect to dynamic lung volumes. (See Results.)

No indications of muscular exhaustion during this very short work were at hand, which is in agreement with studies of muscular function in other muscle groups in myasthenia gravis (34). The analyses also indicate that the original differences in the dynamic lung volumes between observed values and those predicted from only anthropometric variables could not, or only to a minor extent, be directly explained by a decrease in *respiratory force, but that a substantial part* of the effect was mediated via the changes in the vital capacity The close association between respiratory force available and lung volume makes it, from an analytical point of view very difficult to separate these two variables from each other

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CHANGES IN RESPIRATORY MECHANICS IN MYASTHENIA GRAVIS WITH THERAPY

Counteracting Effects of Anticholinesterase

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Abstract. The effects of anticholinesterase, and subsequently sympathomimetics, on respiratory mechanics have been studied in ten patients, with moderate to rather severe myasthenia gravis, one of them being studied separately. A systematic increase in lung resistance was first shown to occur. The latter seemed to increase with larger doses of anticholinesterase while sympathomimetics gave return to the initial values. Subsequently increases in the maximum respiratory pressures ($P_i$max and $P_e$max), the static lung volumes with maximum inspiration included and all dynamic lung volumes were found. Considering the basic disease, the increases in respiratory forces were thought of causal importance for the increased lung volumes. Using the functional relationships found in the untreated state this could also be shown for the change in the vital capacity. For the dynamic lung volumes the changes in the forced inspiratory (FIV) and expiratory volume ($FEV_{1.0}$) were primarily mediated by this dimensional effect i.e. increase in vital capacity. The increase in maximum voluntary ventilation (MVV) was, however, dependent on the dimensional effect to about the same extent as on a more direct muscular effect on the ventilatory forces. These changes are in principle in agreement with existing conditions in healthy controls. The successive deterioration of the disease in one patient and varying degrees of medication made it possible to instructively illustrate both these mechanical interrelationships and important implications for simultaneous slight airway obstructive disease when administering anticholinesterase. Thus, the present results indicate that the clinical improvement in myasthenia gravis reported in other studies after ephedrine medication may to a significant extent be explained by its sympathomimetic action on the smooth bronchial muscles.

Successful treatment of the muscle weakness in myasthenia gravis, by using eserine, was first reported by Walker in 1934 (22). Other investigators demonstrated later the effects of other anticholinesterase drugs (19 23). An improved prognosis has been shown to result from this therapy (12) but it enhances the risk of cholinergic crisis (13 24) in which symptoms from the respiratory system are of great importance (8, 21). This seems to be the case especially with infections (21) and surgical interventions, e.g. thymectomy (8) in which an acute deterioration of the respiratory function is reported to be a major risk (8, 12, 21).

When routine treatment has not had the intended effect, ephedrine has sometimes also been used (3 4) and led to improvement, mainly clinically judged. However no conclusive evidence either of any effect or of the mechanism of the presumed effect has been presented for this type of therapy.

Because of the importance of the respiratory system and the scarcity in the literature of objective information on the effect of treatment on the respiratory mechanics an analysis is obviously needed. The present study is an attempt to give a more detailed evaluation in this respect using a group of myasthenia gravis patients with changes in the respiratory mechanics in the untreated state (15). Further a unique possibility to make a direct study of the respiratory mechanical interrelationships is provided.

MATERIAL

Five women and five men, aged 17–64, with moderate to rather severe myasthenia gravis, were examined. Nine patients are identical with those on whom studies of respiratory mechanics have been made without medication (15).

Patient 10, a 41 year-old man, was studied separately because of large discrepancies both with respect to

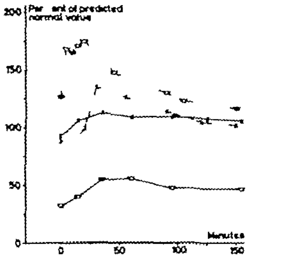


Fig. 1 Influence of Synstigmin on lung resistance ( ) and $P_E$max (——) expressed as percentage of predicted values. Synstigmin (12 μg/kg b. wt.) was given intravenously to patient 2 (○) and intramuscularly to patient 1 ( ).

case history and the respiratory mechanics, which made it very difficult to include him in the group. Thus, besides rather severe myasthenia gravis he also had signs of an obstructive lung disease. It was, however, of the utmost interest to study the effect of anticholinesterase on a patient with both extreme involvement of the respiratory muscles and symptoms of restrictive lung changes. He was included in the group for the analyses summarized in Table VII, as no consideration was taken to the absolute values and the influence of lung resistance had been neutralized. The muscular strength was extremely low at trunk flexion—13% of predicted normal value—but also much decreased at elbow flexion and handgrip—37% and 55% respectively. He also showed symptoms of respiratory distress, ptosis and diplopia.

The patients were compared with the same, healthy control series (16, 17) as in a previous study (15). Intra-individual comparisons were made with the results of the respiratory mechanics in the untreated state of myasthenia gravis (15).

METHODS

The respiratory mechanics studies were made in accordance with procedures described earlier (15) and included determination of *static and dynamic lung volumes, lung resistance maximum expiratory and inspiratory pressures* (P_E*max and* P_I*max*).

Time-response studies. Repeated measurements of the lung resistance and the $P_E$max were carried out on special occasions for 2 1/2 hours after a single injection of neostigmine (Synstigmin®) to patients 1 and 2, administered intravenously to patient 1 and intramuscularly to patient 2, in a dose of 12 μg/kg b. wt.

General procedure. The examinations were carried out in the forenoon in direct succession to a similar study of the respiratory mechanics of myasthenia gravis in the untreated state (15) in which lung resistance was measured as the last step. The patients were either untreated, or anticholinesterase or other medication had been withdrawn at least 12 hours before the start of the first examination. Anticholinesterase was given as a single injection of Synstigmin, intramuscularly or intravenously in a dose of about 12 μg/kg b. wt., except patient 10, to whom 8 mg pyridostigmine (Mestinon®) was given. After administration of anticholinesterase lung resistance was measured with a time interval dependent on the method of administration (intramuscularly to patients 1–8 and 10, 18–36 min after injection, and intravenously to the other subjects, after 8–15 min).

Isoprenaline in the form of dose-aerosol was subsequently administered to all patients except patient 3 directly after measurement of lung resistance. They made 3 to 4 deep inhalations. The patients had earlier practiced the inhalation technique. Fifty mg ephedrine was administered subcutaneously to patient 3. In this patient, therefore, the respiratory mechanics were examined after the longest interval. On the average the lung function studies started about 10 min after administration of sympathomimetics. Lung resistance was the first variable measured except for two patients (nos. 6 and 8) in whom vital capacity was measured first. After lung resistance the $P_E$max and $P_I$max were measured, except for patient 1 in whom $P_E$max and $P_I$max were the last to be measured, 80 and 86 min respectively after injection of anticholinesterase. The whole examination finished with functional residual capacity and other static lung volumes at the latest 90 min after injection of anticholinesterase.

Dose-response studies. The effect of various anticholinesterase doses on respiratory mechanics was examined in patient 10 on different days.

Normal values, relative values, statistical methods and abbreviations as in a previous report (15) were used. Relative difference or change means the difference between the relative value after and before medication.

RESULTS

Effect of anticholinesterase medication

Time-response studies. After Synstigmin was given intravenously to patient 2 an increase of

Table I. *Influence of increased dosage of anticholinesterase on maximum expiratory pressures and lung resistance in patient 10*

	Date	$P_E$max (cm H_2O)	$P_I$max (cm H_2O)	Re (cm H_2O l/sec)
Without medication	Nov 20, 63	21	24	5 1
Pyridostigmine				
2 mg i.m.	Nov 20 63	34	39	7.9
5 mg i.m.	Nov 22, 63	77	33	8.3
7 mg i.m.	Dec. 12, 63	74	34	9.8
8 mg i m.	Mar 12, 65	76	43	10.8
180 mg p.o.	Jan. 25 64	88	62	11 4

Table II. *Differences between observed absolute and relative values after and before medication for lung resistance, maximum respiratory pressures and static lung volumes. The mean intra-individual differences are also given for patients 1–9*

Examination of the respiratory mechanics in the untreated state of myasthenia gravis was made immediately before medication

	Anticholinesterase		Anticholinesterase + sympathomimetics													
	Ra		Ra		$P_E$max		$P_I$max		VC		FRC		RV		TLC	
Pt. no.	cm H_2O l/sec	% NV	cm H_2O l/sec	% NV	cm H_2O	% NV	cm H_2O	% NV	l BTPS	% NV	l BTPS	% NV	l BTPS	% NV	l BTPS	% NV
1	1.4	40	−0.4	−11	26	17	−3	−4	−0.02	0	−0.02	−1	0.09	5	0.07	1
2	0.5	18	0.4	14	−5	−3	17	17	−0.04	−1	−0.09	−3	−0.11	−8	−0.15	−3
3	0.5	18	0.2	−7	91	38	9	−6	0.18	3	0.05	2	0.23	11	0.41	5
4	2.8	88	0.7	22	16	9	17	16	0.29	8	−0.20	−9	−0.37	−32	−0.08	−2
5	0.3	10	0.1	3	59	26	28	25	0.63	21	0.49	12	0.16	6	0.79	10
6	0.9	31	−0.5	17	6	2	3	2	0.46	9	0.07	2	−0.15	−10	0.31	5
7	1.0	40	0	0	37	22	18	15	0.48	11	0.03	1	−0.20	−17	0.26	5
8	2.7	90	0.7	23	10	4	41	34	0.38	8	0.04	1	0.04	1	0.42	4
9	0.9	29	0	0	36	22	11	11	0.47	12	0.26	10	−0.09	−6	0.38	8
Mean	1.2	40	0.1	3	31	15	14	12	0.31	8	0.07	2	−0.04	−6	0.27	4
10	7.2	277	0.2	8	48	20	28	21	2.87	45	−0.28	−6	−0.52	−23	2.33	27

p 0.05 p 0.01

Table III. *Observed absolute and relative values for lung resistance, maximum respiratory pressures, and static lung volumes after medication. The mean intra-individual differences between observed (Obs.) and predicted (Pred.) values are also given for patients 1–9*

	Anticholinesterase		Anticholinesterase + sympathomimetics													
	Lung resistance		Ra		$P_E$max		$P_I$max		VC		FRC		RV		TLC	
Pat. no.	cm H_2O l/sec	% NV	cm H_2O l/sec	NV	cm H_2O	NV	cm H_2O	% NV	l BTPS	% NV	l BTPS	% NV	l BTPS	% NV	l BTPS	% NV
1	4.6	131	2.8	80	154	100	106	124	3.35	93	2.31	86	1.52	92	4.86	93
2	2.8	100	2.7	96	117	73	128	128	4.25	97	3.04	102	1.12	77	5.37	95
3	2.7	96	2.0	71	257	107	135	103	3.04	84	3.49	79	1.84	83	6.88	93
4	4.6	144	2.5	78	72	42	112	102	2.95	81	1.75	75	0.66	56	3.61	78
5	2.9	100	2.7	93	183	82	107	94	5.45	110	4.11	94	2.33	89	7.78	95
6	4.5	155	3.1	107	125	49	77	54	4.39	87	2.81	81	1.28	88	5.67	89
7	3.5	140	2.5	100	97	56	77	65	2.69	65	2.27	90	1.43	121	4.12	83
8	7.2	240	5.2	173	171	75	96	81	3.40	65	2.76	66	2.11	86	5.51	70
9	3.4	110	2.5	81	81	49	99	57	3.30	85	3.16	122	1.84	142	5.14	103
Mean	4.0	135	2.9	98	146	70	100	90	3.87	85	2.86	88	1.57	93	5.44	87
Difference Obs.–Pred.																
Mean	1.06	35	0.1	2	57	30	14	10	0.71	14	0.43	12	0.15	7	0.82	13
10	10.8	415	3.8	146	76	32	43	32	5.65	88	7.14	153	6.02	266	11.65	113

<0.05; $p<0.01$

Table IV *Differences between observed absolute and relative values for dynamic lung volumes after and before medication with anticholinesterase and sympathomimetics. The mean intra-individual differences are also given for patients 1 9*

Examination of the respiratory mechanics in the untreated state of myasthenia gravis was made immediately before medication

Pat. no.	$FIV_{1.0}$		$FEV_{1.0}$		MVV_{40}	
	l BTPS	% NV	*l BTPS*	% NV	*l BTPS*	% NV
1	−0.14	−5	0.08	2	3	3
2	0.02	0	0.09	2	6	17
3	0.18	4	−0.13	−3	8	6
4	0.11	4	0.58	18	6	4
5	0.89	19	0.55	15	21	14
6	0.34	8	0.18	4	23	11
7	0.67	19	0.63	18	16	12
8	0.44	10	0.33	9	21	14
9	0.54	17	0.43	14	39	29
Mean	0.34	8	0.30	9	16	12
10	1.84	33	1.24	26	42	22

$p < 0.05$ $p < 0.01$

lung resistance from about 125% to about 170% of predicted normal mean values was found on the first recording after 5 min (Fig. 1). The increase *remained for 5 min after which the lung* resistance was somewhat lower and reached the initial value about 90 min after the injection. In case 1 who was given an intramuscular dose of Synstigmin the increase of lung resistance occurred after only about 30 min. The increased lung resistance remained for half an hour then a slow decrease occurred. But after 150 min the initial value had still not been reached. P_Lmax showed an increase in both patients to a maximum at the 30 min measurement, with a value only slightly lower 2 1/2 hours after the administration of Synstigmin.

Dose-response studies. Increasing doses of anticholinesterase on different occasions gave an increasing lung resistance in patient 10 (Table I). The highest increase in lung resistance together with the highest increase in maximum respiratory strength was obtained after a high peroral dose of Mestinon®

Lung resistance increased in all patients. The increase was significant ($p < 0.01$ Table II), although with large interindividual variations to a mean value that was higher ($p < 0.05$) than the predicted normal mean value (Table III).

A significant correlation ($r = 0.79$ $p < 0.01$) was at hand between the absolute lung resistance before and after medication ($r = 0.77$ $p < 0.05$ for the relative value).

Effect of anticholinesterase and sympathomimetics

Lung resistance After sympathomimetics had also been administered, lung resistance decreased in all patients to approximately the level before anticholinesterase medication (Table III).

Maximum respiratory pressures The P_Lmax increased in all patients except patient 2 while the P_Imax increased in seven but not in two of the three patients with the highest initial relative values. An increase in either inspiratory or expiratory force occurred in all patients. The increase in P_Emax for the group was significant ($p < 0.01$) and for P_Imax probably significant ($p < 0.05$ Table II). P_Emax was still lower ($p < 0.01$) than the predicted normal mean value, while P_Imax was no longer systematically lower than the predicted normal mean value in the group (Table III)

No significant correlations were found between the initial levels of P_Imax or P_Lmax and the changes in these variables ($r = 0.52$ and $r = 0.12$, respectively).

Table V *Observed absolute and relative values for dynamic lung volumes after medication with anticholinesterase and sympathomimetics. The mean intra-individual differences between observed (Obs.) and predicted (Pred.) values are also given for patients 1 9*

Pat. no.	$FIV_{1.0}$		$FEV_{1.0}$		MVV_{40}	
	l BTPS	% NV	*l BTPS*	% NV	*l BTPS*	% NV
1	2.95	99	2.68	100	123	115
2	4.16	112	3.91	108	168	1 4
3	4.98	96	4.12	94	184	103
4	2.67	87	2.65	85	78	62
5	4.69	102	4.14	114	119	87
6	4.43	105	3.52	82	140	75
7	2.69	77	2.69	74	103	81
8	3.19	72	2.14	58	95	62
9	3.06	94	3.15	97	112	83
Mean	3.65	94	3.22	90	124	88
Difference Obs. Pred.						
Mean	−0.24	−6	−0.38	−10	−17	12
10	3.38	60	2.36	50	95	50

Static lung volumes. There was an increase for the group in vital capacity ($p < 0.01$) and total lung capacity ($p < 0.05$ Table II). The observed absolute and relative values for vital capacity were still lower ($p < 0.01$ and $p < 0.05$ respectively) than the predicted normal values of the group (Table III). The observed mean value of total lung capacity was also still lower ($p < 0.01$) than the predicted normal mean value for the group (Table III). No significant mean changes were found in functional residual capacity and residual volume (Table II).

Dynamic lung volumes. The effect of medication on $FEV_{1.0}$ and MVV_{40} was significant ($p < 0.01$) while it was probably significant ($p < 0.05$) with regard to $FIV_{1.0}$ (Table IV). The relative increase in these three variables was of the same magnitude as the relative increase in maximum respiratory force and vital capacity (Table II). No significant difference for the group was found between these three ventilatory capacity tests after medication and the predicted normal mean values (Table V).

Table VI. *Differences (Δ) between absolute values for lung function variables after medication (anticholinesterase + sympathomimetics, final values) and before medication (initial values). These differences (A) have been corrected with regard to the change in causal variables found in a normal series (16, 17) (see below under 1) and (B) with regard to vital capacity also in the untreated myasthenia series (15) (see below under 2)*

Lung function variables	Final values (mean)	Differences A Final-initial values (mean)	Differences B Final-initial corrected values (mean)
VC	3.87	0.31	0.25
FRC	2.86	0.07	0.19
RV	1.57	−0.04	0.04
TLC	5.44	0.27	—
FEV	3.22	0.30*	0 08
FIV	3.65	0.34	0 03
MVV_{40}	124	16	8
VC	3 87	0.31	0.12 (2)

Equations for correction

1 ΔVC 0 0041 $\Delta P_I max$

$\Delta FRC = -0\ 0043\ \Delta P_E max$

$\Delta RV = -0.0026\ \Delta P_E max$

$\Delta FEV = 0\ 0019\ \Delta P_E max + 0.55\ \Delta VC - 0.14\ \Delta Re$

$\Delta FIV = 0\ 0052\ \Delta P_I max + 0.775\ VC$

$\Delta MVV_{40} = 0.11\ \Delta (P_I max + P_E max) + 13.0\ \Delta VC - 3\ 6\ \Delta Re$

2. ΔVC 0 14 $\Delta P_I max$

p 0.05; p 0.01.

Table VII. *Influence of relative changes in maximum respiratory pressure (D $Pmax_{rel}$) and vital capacity (D VC_{rel}) on relative changes (the intercept) in static and dynamic lung volumes*

The analyses have been made as regression analyses with the relative changes in lung volumes as regressands and relative changes in Pmax or VC, the latter only for dynamic lung volumes, as regressors. Intercept A = the relative difference after and before medication, intercepts B and C = the difference after consideration has been taken to the *changes in relative Pmax or relative VC (= 10)*

	Intercept A		Intercept B		Intercept C	
Regressand	M	S.E.	M	S E.	M	S E.
D VC_{rel}	11.6	4 1	6 2	6.1	—	—
D FRC_{rel}	0.9	2.1	−2.1	3 2	—	—
D RV_{rel}	−8 1	4 5	−14 2	7 5	—	—
D TLC_{rel}	6 1	2 5	3 7	3 6	—	—
D $FEV_{1.0 rel}$	10 6	3 1	9.6	5 1	4 7	2 5
D $FIV_{1.0 rel}$	10.9	3.5	5 4	4 9	2.8	2.3
D $MVV_{40 rel}$	13 3	2.6	7.5	5 9	9 9	3 1

$p < 0.05$ $p < 0.01$ $p < 0\ 001$

Explanatory value of the maximum respiratory forces and the vital capacity. It has already been possible to study these relationships indirectly (15) but by means of medication a more direct study of the effects of the causal variables included is possible. In addition more detailed analyses of these interrelationships were carried out on the patient with the most severe affection of his respiratory muscles (pat. 10).

First, the changes found for the static and dynamic lung volumes were compared with those predicted from the simultaneous changes in causally related variables and the functional relationships established in a normal series (16, 17) and, for vital capacity also from the myasthenia series (15) in the untreated state. Table VI shows that for the changes in the *static lung volumes* the changes in maximum respiratory forces seemed to have no certain influence according to the functional relationships found in normal subjects. On the other hand, the change in the maximum inspiratory force could explain the change in vital capacity if the functional relationship based on the myasthenia series was used. For the *dynamic lung volumes* the systematic increase after medication could, however be explained by the aggregated effect of Pmax, vital capacity and lung resistance according to the functional relationships in normal subjects (Table VI).

Table VIII. *Variations of the MVV with different values for $P_E$max and $P_I$max VC and lung resistance in patient 10 on different occasions*

Note that MVV was lowest when lung resistance was highest, even though $P_I$max was at its highest
Pred. A – values predicted from anthropometric data only (16, 17). Pred. B – values for MVV predicted from anthropometric variables, actual Pmax, VC and lung resistance (16, eq. 12 in Table 29)

Experimental situation	Examination	$P_E$max (cm H_2O)	$P_I$max (cm H_2O)	VC (l BTPS)	Lung resistance (cm H_2O/l/sec)	MVV (l BTPS/min)	MVV (Pred. B) (l BTPS/min)
Normal values (Pred. A)		241	135	6.4	2.6	190	
Without medication	A	87	39	5.5	4.8	85	131
Without medication	B_1	45	25	3.2	5.3	53	92
Without medication	C	28	15	2.8	4.0	53	97
With anticholinesterase	B_2	88	62	3.2	11.4	33	64
With anticholinesterase +	B_3	89	53	3.5	9.0	52	86
sympathomimetics	C_2	76	43	5.7	3.8	95	142

Further analyses of the influence of Pmax and/or vital capacity on the change in lung volumes were made with multiple regression analyses using the intra-individual changes in the whole myasthenia gravis series (Table VII). The relative change in the vital capacity had a significant influence on the relative changes in $FEV_{1.0}$ and $FIV_{1.0}$. Otherwise no significant parameters were found, although it should be pointed out that the changes in the other lung volumes—the regressands or intercepts—lost their significant values.

Counteracting effects of anticholinesterase on lung resistance and respiratory muscle forces. The examinations of patient 10 were made on various occasions both with and without anticholinesterase medication—in one study combined with inhalation of isoprenaline (Table VIII). There were marked variations in lung resistance, respiratory force and vital capacity. It was therefore possible to make a direct study of the effect on the MVV of these different constellations of causal factors.

At first examination (A) a much decreased Pmax, moderately increased lung resistance, but a vital capacity only slightly depressed were present. The resulting MVV was about 45% of the predicted normal mean value. Successively decreased respiratory force and vital capacity were later recorded (B and C_1) giving a further decreased MVV than on occasion A. On the last occasion (C_1) the maximum respiratory pressures were extremely small—about 10% of the predicted normal mean value—while relative vital capacity was slightly more than 40%. The largest decrease in vital capacity occurred between examinations A and B_1. The relatively small difference in MVV between occasions B_1 and C_1 may be explained by a somewhat lower lung resistance on occasion C_1.

After administration of anticholinesterase (B_2) an approximate doubling of the respiratory force occurred. In spite of this fact MVV was further diminished to a very low value. Simultaneously a marked rise in lung resistance and an unchanged vital capacity were recorded. At the same time the patient experienced a general improvement and also a loss of the respiratory distress. On occasion B_3, with almost the same respiratory force and slight decrease in lung resistance, a slight increase in vital capacity was recorded together *with a MVV equivalent to the initial value without medication* (B_1). After a near normalization of lung resistance obtained by sympathomimetics (C_2), vital capacity and MVV increased to values equivalent to those obtained on occasion A, when a comparable respiratory force was available. The predicted values for MVV were consistently higher than the observed values, also when the maximum respiratory pressures, vital capacity and lung resistance were accounted for (15, 16, 17) (Table VIII).

DISCUSSION

Deranged respiratory mechanics without obvious clinical signs have been demonstrated in untreated

myasthenia gravis in an earlier study (15). Investigations of the respiratory mechanics in myasthenia gravis patients after medication have not been reported, though in many clinical studies respiratory complications after anticholinesterase have been demonstrated (8, 12, 24, 25). Cholinergic crisis is particularly hazardous in patients with weak respiratory muscles (21).

Anticholinesterase may have counteracting effects on the respiratory system. Parallel to a favourable nicotine-like effect on striated musculature (6) anticholinesterase may, via acetylcholine, also have a constricting effect on the smooth bronchial musculature (6), giving an increased lung resistance.

By means of bronchodilators it is possible to control the latter effect to different extents. Hence, a unique experimental situation exists with regard to variations in static and dynamic lung volumes and their dependence on different causal factors. The magnitude of the influence of these variables on lung volumes has previously only been estimated through indirect analyses of intra and inter-individual variations in healthy subjects (11, 14, 16, 18).

The primary effect attempted in the present study was to increase the force of the respiratory muscles in the patients. With unchanged flow resistive properties in the lungs—obtained after sympathomimetics—it is reasonable to expect any change in the static and dynamic lung volumes studied to be primarily due to the increased respiratory force. Further analyses may then be directed towards discovering the ways in which the increases found in the lung volumes were achieved.

Lung resistance. An increase in lung resistance was obtained after anticholinesterase medication in all patients. This was not possible to detect clinically as all patients experienced a subjective improvement and did not report any symptoms of impaired respiration. Even patient 10, who on one occasion had a doubling of lung resistance in conjunction with a diminished maximum voluntary ventilation to a very low value, only experienced the improved general muscle strength although a disturbed O_2-exchange was most probably at hand.

It is important to note that the increased muscle strength thus had a stronger positive effect on the respiratory sensation than the negative effect of increased airway resistance. The nicotinic stimulating action of acetylcholine on the neuromuscular transmission can evidently conceal the muscarinic action on the respiratory mechanics. This may conceivably explain why increasing doses of anticholinesterase may lead to a cholinergic crisis with paresis of the respiratory muscles without any previously observed secondary effects of the muscarinic type (21).

Clinical observations of myasthenia gravis patients after thymectomy indicate, however, that secondary effects of the muscarinic type in this situation are prominent when an overdose of anticholinesterase is given (8). It has also been pointed out (10) that close attention to clinical signs of muscarinic intoxications is of paramount importance during treatment of myasthenia gravis. Clinical observations in healthy subjects exposed to insecticides with an anticholinesterase effect also showed that the effects of the muscarinic action usually appeared before the inhibiting nicotinic action on striated muscle (7).

Earlier it was thought that the respiratory muscles in myasthenia gravis patients could easily be overdosed with an impairment of the ventilatory capacity while other muscles were still underdosed (20). The results of this investigation imply that an impairment in ventilatory capacity after anticholinesterase therapy may, in some patients, be due to an increased lung resistance, i.e. a secondary effect of the muscarinic type.

Ephedrine has also been used to treat myasthenia gravis (3, 4). It has been thought to increase the effect of the specific anticholinesterase drugs (1, 5). According to Osserman (12) ephedrine did not effect any further increase of the muscle strength beyond that achieved by pyridostigmine tested with quantitative methods.

Another sympathomimetic, isoproterenol, is widely used by direct inhalation for the symptomatic treatment of bronchial asthma and has been used in this investigation. As isoproterenol stimulates beta-adrenergic receptors (6) it relaxes the smooth muscles in the walls of the bronchi and thereby counteracts the muscarinic action of acetylcholine induced by anticholinesterase. The effect on lung resistance was marked in this investigation and the values of lung resistance rapidly returned to the initial values. The clinical improvement in myasthenia gravis patients treated with ephedrine may therefore to a significant extent be

explained by the sympathomimetic action on the smooth bronchial muscles.

Static lung volumes. The increase of vital capacity may be obtained by means of an increased end-inspiratory volume and/or decreased end-expiratory volume. The fact that the end-inspiratory level was about 80% of the predicted value together with a normal residual volume suggested that any increase in vital capacity would primarily be obtained by means of an increased end-inspiratory level. The results after medication with an increase in total lung capacity together with an unchanged residual volume confirm this assumption. The agreement between the increase in the vital capacity and that predicted from the regression coefficient for $P_I$max in the untreated myasthenia gravis patients (15), larger than would be expected from healthy subjects (16), provides further support for the contention that the change in the inspiratory muscle force is of direct causal importance for the change in vital capacity.

In contrast to the effect on the maximum inspiratory level, anticholinesterase did not change the maximum expiratory level, i.e. the residual volume was unchanged. This might be consistent with the conceptions that the limiting mechanisms of the lower volume extreme are operating in the chest wall and/or the lungs (9). It has, however, been shown that inter-individual variations in expiratory muscular strength marginally influence the residual volume (15) and that contraction of the antagonist muscle contributes to the static balance of forces limiting the lower volume extreme (1, 2). The present results did not confirm any effect of the expiratory force as found in the healthy series quoted (15). The only moderate change in $P_E$max and the small series should, however, be considered.

The unchanged functional residual capacity decreased in the untreated state, does not indicate any muscular effect.

Dynamic lung volumes. The changes produced by medication in the dynamic lung volumes, regressands, and the causal regressors considered have provided the interesting and unusual possibility of studying directly the interrelationships between these regressors and regressands.

Variations in dynamic lung volumes may theoretically be considered to be due to variations in the generating and/or opposing forces working on the lung volume available. The intra-individual relationships of these factors and their general relevance with respect to inter-individual variations in different dynamic lung volumes have been described in detail (5).

Maximum respiratory pressures, lung resistance and vital capacity have been used as approximate expressions for inter-individual variations in these factors. Assuming full cooperation, i.e. maximum effort during the studies, significant to highly significant and mostly very marked influences of maximum respiratory pressures, lung resistance and vital capacity have been found in healthy subjects for the dynamic lung volumes except that lung resistance did not affect the inter-individual variations in forced inspiratory volume in one second ($FIV_{1.0}$) (16).

At first glance one might conclude the existence of an independent influence from each of these regressors on the regressand, e.g. a larger vital capacity should give a larger $FIV_{1.0}$ and MVV with no changes in the other causal factors. Theoretically such assumptions, i.e. no relationships between the regressors, are inherent in the conditions present when carrying out multiple regression analyses. In practice, however, this situation is very difficult to achieve in the respiratory system as the variables are interdependent. To some extent this disadvantage may be abolished by knowledge of these assumptions and physiological reasoning.

In this respect the present study provided some instructive illustrations. After medication an increase both in vital capacity and ventilatory capacity was obtained with the increase in respiratory force. In addition, this change in the dynamic lung volume could be predicted when the functional relationships for the causal regressors, as obtained from the controls, were used.

The changes in the dynamic lung volumes for the group studied gave possibilities to illustrate the risks of a too "static" use of the statistical analyses of this model. Thus, if one tries to analyse the apparently immediate cause of the changes in the regressands by means of intercept analyses, the change in vital capacity seems to be the sole responsible factor for the increase in $FIV_{1.0}$ and $FEV_{1.0}$. However, as we are in the unique position of knowing that the increase in the vital capacity from a physiological and logical point of view was produced by the change in muscular force, lung resistance unchanged, the

increase in $FIV_{1.0}$ and $FEV_{1.0}$ must be interpreted as being primarily dependent on the increase in muscular force.

For the group the intercept analyses could not indicate any single causal factor for the increase in MVV although both the respiratory force and the vital capacity increased. As presented above the latter variable was of causal importance for the changes in single forced inspirations and expirations, respectively and both such processes are repeatedly included in MVV The explanation may be found both from statistical and physiological considerations. Hence, in the controls (16) the dependence of the variations in MVV on the maximum respiratory pressures was more pronounced than for $FEV_{1.0}$ and $FIV_{1.0}$ and approached that of vital capacity In a primarily neuromuscular disease which is characterized by a decreased *isometric strength*, at least similar if not more pronounced conditions should be present and thus also influence the results of the analyses. Considering the conditions required for the multiple regression analysis the simultaneous and equal changes in vital capacity and maximum respiratory forces and their interrelationship may explain the loss of significant influence for each of these variables with regard to the change in MVV

The prediction of lung volumes in the diseased state using regression equations obtained from normal controls is limited when treating data obtained from patients with very severe involvement of the muscular force as shown in patient 10 (Table VIII). When comparing observed and predicted values consideration must be taken to the residual standard deviation. Furthermore in the extremes of a regression line the confidence limit for the mean value of the regressand deviates strongly so the prediction of lung volume may be highly uncertain for a patient with such extreme values for the regressors as patient 10.

The various numerical constellations of causal and resulting variables for this patient, however confirmed earlier intra- and inter-individual findings obtained with respect to respiratory mechanical interrelationships (11 14 16, 18) It should especially be observed that for a large decrease in the respiratory forces the vital capacity was only moderately diminished. Only after a further deterioration of the disease with respiratory forces at extremely low levels was there a marked reduction of vital capacity

The effect of sympathomimetic medication was instructively demonstrated in patient 10 with obstructive lung disease. On administration of anticholinesterase the muscarinic actions exerted a predominating influence on the ventilatory capacity Not until this effect was eliminated by sympathomimetics was the desired favourable effect of increased respiratory strength obtained.

CONCLUSIONS

The effect of medication on the respiratory mechanics may be difficult to assess in myasthenia gravis as the subjective experience of the general improvement in muscular strength can conceal the effect of increased lung resistance. It is therefore important to be aware of the counteracting effects on the respiratory mechanics caused by anticholinesterase medication. Furthermore, in myasthenia gravis anticholinesterase and bronchodilator medication provide unique possibilities for the study of respiratory mechanical interrelationships, primarily that of increased respiratory muscular force.

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URINARY PROTEINS AND PLASMA RENIN ACTIVITY DURING TOTAL STARVATION

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Abstract. Fifteen overweight patients, aged 19–65 years, have been treated by total starvation for 2–9 weeks. I six patients urinary protein patterns of tubular type ere found as shown by paper electrophoresis and gel filtration, accompanied by increased excretion of ribonuclease. In tw cases, followed weekly potassium substitution therapy normalized the tubular patterns. The results indicate that starvation induces reversible tubular proteinuria, probably secondary to potassium deficit. Plasma renin activity in the three patients studied showed varying changes during and increased values after the starvation period in tw of them.

Prolonged starvation gives rise, among other things, to metabolic acidosis, decreased excretion of nitrogen, amino acids, sodium and potassium (20, 22). Serum potassium and total body potassium also show a moderate decrease (7). A decrease in acidifying ability (21) and glomerular filtration rate is also reported (20). In congenital and acquired hypokalaemia a typical pattern of the urinary proteins usually appears, presumably a sign of tubular dysfunction (2, 4 16).

The decrease in total body potassium is probably due to the minute potassium conservation ability of the kidneys, in contrast to their great ability to excrete excess potassium. Renal tubular dysfunction might further impair potassium conservation. The present investigation was undertaken to find out whether prolonged starvation will induce a tubular" pattern in the urinary proteins as a sign of tubular impairment. Increased urinary potassium losses may also be secondary to hyperaldosteronism. Some results (9) suggest a hyperaldosteronism during starvation. Therefore a study of plasma renin activity (PRA) which is known to be increased during secondary hyper aldosteronism (14), was included.

MATERIAL AND METHODS

The urines from eight men, aged 27–65 years (cases 1 8), and seven women, aged 20–56 years (cases 9–15), who were treated for overweight by total starvation, ere studied. T patients (cases 6 and 11) were treated t ice with an interval of 6 weeks. A detailed description of the starvation procedure has been given lsewhere (13). The periods of starvation varied bet een 2 and 9 week

I an initial study of twelve cases 24 hour urine volumes were collected only once or twice during the period of starvation. In second study 24-hour urines were collected every week during the starvation hree women aged 31–43 years (cases 13-15). Data on 1 1 patients are given in Table I.

Protein (17), ribonuclease (12) and α-ami acid N (21) were determined in all urines. The urines were concentrated in collodium tubings by ultrafiltration under negative pressure to small volume and separated in paper electrophoresis (16, 18). Urinary concentrates were also separated by gel filtration on Sephadex G-100. The data for potassium in serum were obtained from the hospital charts. In the three cases, followed weekly creatinine (10) was also determined in urine and excretion of protein, ribonuclease and α-aminoacid-N was expressed as mg/g creatinine because of difficulties in obtaining complete 24-hour urine samples. PRA was determined by the method of Boucher (3), modified by Castenfors (5).

RESULTS

In three of the first twelve patients (cases 7 and 12) there w s an increase of total urinary protein and ribonuclease during starv tion, and paper electrophoretic examination showed that the urinary protein patterns were of a tubular type w th increases predominantly in the α- β- and γ-regions (Fig. 1 5 1 6). Separations by gel filtration showed that there was mainly an excre tion of proteins with molecular weights less than that of albumin (Fig. 2). In a fourth patient (case

Table I. *Weight reduction, starvation period and tubular proteinuria*

Case	Sex	Age (y.)	Weight reduction (kg) Period 1	Weight reduction (kg) Period 2	Starvation (d.) Period 1	Starvation (d.) Period 2	Comments	Tubular proteinuria
Initial study								
1	♂	26	14.9		21			
2	♂	46	7.9		19		Hypertension	
3	♂	65	17.7		33		Hypertension	
4	♂	61	12.9		29			
5	♂	27	9.4		20			
6	♂	40	10.3	14.4	21	29		
7	♂	44	18.9		27			
8	♂	37	24.5		61			
9	♀	47	10.3		23		Diabetes m.	+
10	♀	19	10.1		23			
11	♀	41	10.6	8.5	23	22		+
12	♀	55	14.6		37		Hypertension	+
Second study								
13	♀	31	21.9		60		Nephrolithiasis	
14	♀	43	29.0		53		Hypertension	+
15	♂	39	18.4		52		Asthma	+

11 Fig. 3), who was twice subjected to starvation with a normal feeding period in-between, it was observed that in the first period there was an increase of protein excretion and proteinuria of a tubular type. During the normal feeding period her urinary protein pattern was normalized. During the second starvation period, when potassium was given, the protein pattern was once again of a tubular type, but this time protein excretion decreased.

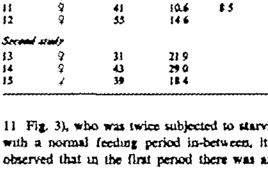


Fig. 1. Paper electrophoretic patterns for urinary proteins. Protein excretion day given in parentheses. (*1*) Normal man (70 mg). (*2*) case 6 (85 mg). (*3*) Case 10 (97 mg). (*4*) Case 4 (64 mg). (*5*) Case 7 (282 mg). (*6*) Case 12 (286 mg).

The three patients who were followed weekly are presented in Figs. 4–6. In case 13 (Fig. 4) α-aminoacid-N and ribonuclease decreased and no change in the excretion of protein was found. In this case no sign of tubular proteinuria was found during the whole starvation period (Fig. 7). At the end of the starvation period of 40 days this patient had an attack of nephrolithiasis which resulted in haematuria, and after that no reliable values for protein excretion could be obtained.

In cases 14–15 (Figs. 5 and 6) there was an increase in the excretion of protein, α-aminoacid-nitrogen and ribonuclease within 10 days from the beginning of the starvation. There were also changes in paper electrophoretic patterns (Fig. 7) to a tubular type with increase mainly of the β- and γ-proteins, and changes in the gel filtration patterns (Fig. 8) of tubular type.

In these cases proteinuria persisted until potassium was administered. Both patients received sodium bicarbonate before they were given po-

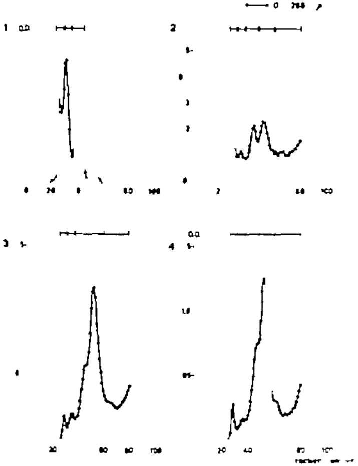


Fig. 2 Gel filtration patterns. Sephadex G-100, 95 2.5 cm, 0.1 M phosphate 0.5 M NaCl, pH 6.5. Fraction volume 5 ml. *(1)* Normal serum. *(2)* Normal urinary proteins. *(3)* Urinary proteins from case 12 *(4)* Urinary proteins from case 7

tassium, but without any effect on the proteinuria. In case 14 (Fig. 5) a decrease in total protein and ribonuclease excretion and normalization of the gel filtration and paper electrophoretic patterns were not achieved until 6 g of potassium chloride/day was given. When the potassium chloride was decreased to 3 g daily there was again a slight increase of the excretion of ribonuclease and protein, which was of the tubular type. In case 15 the administration of 3 g of potassium chloride/day decreased protein excretion, but also in this case the electrophoretic patterns were of the tubular type at the end of starvation.

According to ^{40}K measurements, cases 14 and 15 lost 28 and 22% respectively of their initial potassium content during the first 14 days of starvation, while case 13 did not lose any potassium during the same period.

PRA was only studied in cases 13 14 and 15 (Fig. 9). They all show individual patterns. Case 13 shows a normal initial and gradually increasing value. Case 14 shows an increased initial value followed by decrease during starvation and an increase after the end of the starvation period. Case 15 shows normal values, with a tendency to increase after the end of the starvation period.

DISCUSSION

The starvation period induced different changes in urinary protein excretion in this study Some cases showed an increased, some an unchanged, and some even a decreased urinary protein excretion. In all cases with an increase in protein excretion, however paper electrophoresis showed the proteinuria to be of tubular type, verified by

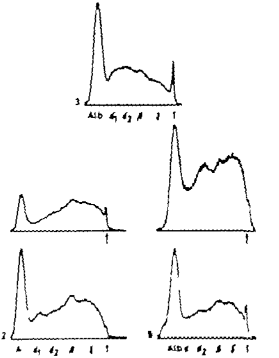


Fig. 3. Paper electrophoretic patterns for urinary proteins from case 11 (mg protein/day). (*1*) First starvation period. After 21 days (116 mg). (*2*) First starvation period. Two days after end of starvation (129 mg). (*3*) Before second starvation period (115 mg). (*4*) Second starvation period. After 20 days (95 mg). (*5*) Second starvation period. Three days after end of starvation (48 mg).

gel filtration studies. Mainly low molecular weight proteins were excreted. A parallel increase in the excretion of ribonuclease also indicates that the proteinuria was of tubular type, as it has been shown that the excretion of this low molecular weight enzyme is increased in tubular disorders (11, 18). Further evidence of tubular dysfunction was the increased excretion of α-aminoacid N in the cases with tubular proteinuria.

In two patients, who were followed weekly, the proteinuria was reversible after oral potassium administration, which suggests that the potassium deficit is of importance for the development of proteinuria. This is also supported by the initial losses of potassium in these two cases compared with the third case, who did not lose any potassium during the same period. Of the six patients who developed tubular proteinuria five were women. It has been shown that women have a tendency to lose more potassium than men (13). This also supports the importance of the potassium deficit as a cause of the tubular proteinuria.

These patterns are similar to earlier published patterns in hypokalaemia due to other diseases (2, 4, 16) but not to patterns found in typical cases of chronic cadmium poisoning with marked proteinuria. They are, however, similar to patterns found in workers exposed to cadmium, but with moderate proteinuria, without any other signs of renal disease (Piscator, unpublished data). In chronic cadmium poisoning the decrease in the reabsorption of proteins is thought to be due to a decrease in the activity of certain enzymes (19). The present study indicates that potassium is of importance for the reabsorption of protein in humans. This agrees with findings in animal experiments (15). Potassium depletion will affect the human kidney and cause an increase also in the excretion of renal tissue proteins, indicating cellular damage, and it has recently been shown that the administration of potassium will normalize this excretion (2).

An interesting aspect of the tubular proteinuria found in this study is the similarity to the protein

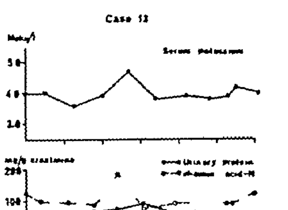


Fig. 4. Serum potassium, urinary excretion of protein, α-aminoacid-N and ribonuclease in case 11.

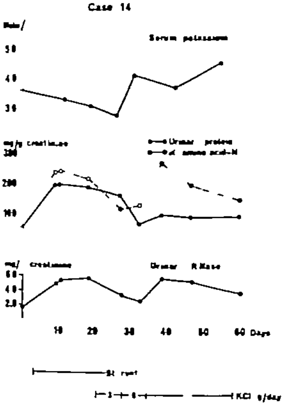


Fig. 5. Serum potassium, urinary excretion of protein, α-aminoacid-N and ribonuclease in case 14

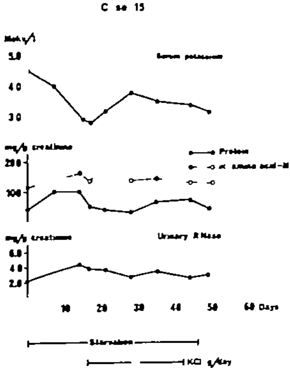


Fig. 6. Serum potassium, urinary excretion of protein, α-aminoacid-N and ribonuclease in case 1

uria found in the endemic Balkan nephropathy a disease which in certain areas of the Balkans has affected a large part of the populations (8). This disease gradually develops later into a tubular syndrome and ends in a uraemic sta e. An early sign of the disease is decrease in acidifying ability The cause of the disease is not known, but the primary effect seems to be localized in the part of the tubules first affected in potassium deficiency

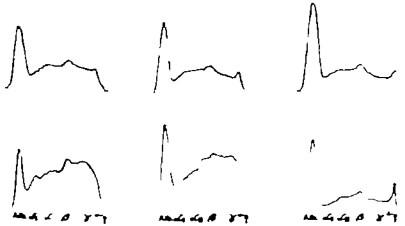


Fig. 7 Paper electrophoretic patterns of urinary proteins from cases 13 14 and 15 (*1*) Case 14 before, (*2*) after 10 days starvation. (*3*) Case 15 before, (*4*) after 7 days starvation. (*5*) Case 13 before, (*6*) after 10 days' starvation.

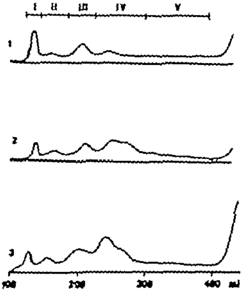


Fig 8 Gel filtration patterns of urinary proteins from cases 14 and 15 Sephadex G-100, 95 2.5 cm, 0.1 M Tris-HCl-0.5 M NaCl, pH 8.0. UV-absorption at 254 mμ (*1*) Case 15 before starvation. (*2*) Case 15 after 7 days of starvation. (*3*) Case 14 after 10 days of starvation.

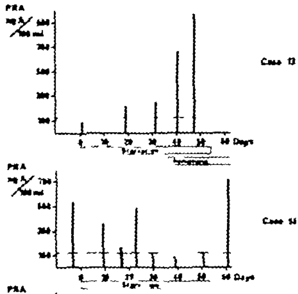


Fig 9 PRA in cases 13, 14 and 15

A tubular pattern may be found without a corresponding increase in total urinary protein and, in such case, may not be indicative of tubular dysfunction. It was shown (Fig. 3) that during the second starvation period, when potassium was given, there was actually a decrease of total protein excretion, but tubular patterns were found. The explanation may be that there is either a specific increase in reabsorption or a decreased filtration of the higher molecular weight proteins.

PRA increased in one case, was normal in one and decreased in the third during the starvation. In two patients PRA increased at the beginning of the refeeding period. This is not in agreement with a recent study (6) on female obese subjects, who showed a marked increase in plasma renin after ten days of total fasting and a decrease after refeeding. The latter study however is not comparable with the present study in which the fasting period was of much longer duration and only a few renin determinations were made during the first ten days of the starvation period.

Potassium deficit is a stimulus to renin release, as shown by a recent study in potassium-depleted dogs (1). This, however does not seem to be of importance in the present study in which there was no correlation between plasma renin and potassium deficit in the three patients studied.

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Kabikinas in standard dosage enables thrombolytic routine treatment of deep venous thrombosis

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Initial dose With an initial dose of 600,000 IU of Kabikinas dissolved in 100—300 ml of 5 per cent glucose or physiologic saline solution — administered for 30—60 minutes — a sufficiently high concentration is obtained to initiate a thrombolytic effect.

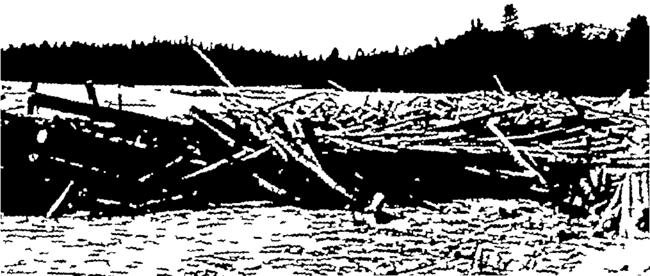
Maintenance dose As a maintenance dose 100 000 IU of Kabikinas per hour are given during the following 72 hours. The treatment can be continued for a further 1—3 days if a clinical effect has been obtained, but phlebographic control has not shown satisfactory dissolving of the thrombus.

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Acta med. scand. Vol. 190 pp. 527–529 1971

A SIMPLIFIED POINT SCORE SYSTEM FOR THE ELECTROCARDIOGRAPHIC DIAGNOSIS OF LEFT VENTRICULAR HYPERTROPHY

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Abstract. A simplified point score system for the ECG diagnosis of left ventricular hypertrophy (LVH) has been tested on 470 autopsies. The total heart weight, adjusted for sex and body length, was used as an indicator of LVH. Patients below 50 years were excluded. A score of more than 2 points strongly indicates an increased heart weight (HW). Forty-two patients scored more than 2 points, 39 (93%) having increased HW, 3 (7%) borderline HW and none normal HW. A score of 1 or 2 points suggests an increased HW. 119 patients having this score, 64 (54%) an increased HW, 42 (35%) borderline HW and 13 (11%) normal HW. A score of 0 point should be interpreted with caution. If the ECG shows no sign of myocardial infarction (MI) or right ventricular hypertrophy (RVH), and ischemic heart disease can be ruled out, this score indicates normal HW. Left bundle branch block (LBBB) suggests an increased HW. Forty-five patients had LBBB, 34 (76%) having increased HW.

Combinations of different ECG criteria in the diagnosis of left ventricular hypertrophy (LVH) have been proposed by several authors (4, 12, 13). *In addition a point score system has been suggested* (9). Both of these approaches have been rather complicated, resting on a series of criteria which may be difficult to remember. Previously we have tested the accuracy of the usual ECG criteria of LVH (11). Based on this and earlier studies (1, 2, 8), we now propose a simplified point score system (Table I). This system is based on only two, well-known ECG criteria. Originally we included ST-T changes in our point score. This has now been excluded, because these changes were difficult to measure. We also found that ST-T changes seldom occurred without one or both of the other criteria being positive. The aim of the present study has been to evaluate this simplified point score system on a large number of unselected autopsies.

MATERIAL AND METHODS

The study comprises consecutive autopsies performed on patients dying in Medical Department IX, Ullevål Hospital, Oslo, during 1966–1969. All patients, 50 years or older, who had an ECG recorded at least once during the last 6 weeks prior to death, were included.

The patological examinations were performed in the Department of Pathology, Ullevål Hospital. The HWs were registered according to the autopsies as recorded by the different prosectors. The HWs were compared with the mean heart weights ($\bar{X}$) of Zeek (14), as adjusted for sex and body length. The patients were divided into four groups:

Extensively increased HW: HW more than twice the mean of Zeek's, HW > $\bar{X}$ + 100%. *Increased HW*: HW = $\bar{X}$ + 50–100%. *Borderline HW*: $\bar{X}$ + 2 S.D. < HW < $\bar{X}$ + 49%. *Normal HW*: HW < $\bar{X}$ + 2 S.D.

The following features were also registered. a) The most usual causes of LVH, i.e. aortic stenosis, hypertension and MI. b) Predominant RVH, i.e. free right ventricular wall ≥ 5 mm and more than one-third of the free left ventricular wall. c) The voltage of S_{max} and R_{max} in the precordial leads (upstrokes and downstrokes measured *according to Wilson et al. (12)*). *d) The duration of the* ventricular activation time, i.e. from the onset of the QRS to the peak of the *R* wave (V_5–V_6) (measured to the nearest 0.01 sec). e) Bundle branch block (BBB), i.e. QRS ≥ 0.12 sec. f) Signs of RVH in the ECG (7).

All ECGs were recorded on an Elema Schonander Mingograph 4-B with a paper speed of 50 mm/sec. Every ECG should be accompanied by a test signal, 1 mV equalling 10 mm. All ECGs, except BBB, were scored according to voltage and ventricular activation time (Table I).

RESULTS

Altogether 470 patients were studied (Table II).

Normal HW was seen in only 112 patients (26%). If the borderline group was registered as normal, still 46% of the patients had increased HW. LBBB was seen in 45 patients, 34 (76%)

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TREATMENT OF HYPERTENSION WITH PROPRANOLOL AND HYDRALAZINE

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Abstract. The effects on arterial pressure of long-term treatment with propranolol and hydralazine have been determined in 17 hypertensive patients (5 with renovascular hypertension and 12 with essential hypertension). The treatment reduced blood pressure in all 17 patients during 28 weeks of treatment (average); daily dose of propranolol was 320 mg (average) and of hydralazine 137 mg (average). Few side-effects occurred but treatment had to be withdrawn in one patient because of tiredness. The results are compared to those obtained in a group of 82 hypertensive patients, who received propranolol as the only treatment. It is concluded that the combined use of the two drugs may be purposeful not only to achieve improved blood pressure control but also to diminish the side-effects of each drug.

Several reports have been published about the usefulness of adrenergic beta receptor blocking agents as a means of controlling hypertension (1, 3, 5, 7). The most widely used drug in this respect is propranolol, which has proved capable of lowering elevated blood pressure both when used alone (1) and in combination with diuretics (7) or methyldopa, guanethidine and bethanidine (5).

It is generally agreed that the reduction of cardiac output is the most important mechanism by which arterial pressure is lowered during treatment with beta receptor blocking drugs. Additionally there is a slight increase of peripheral resistance even during long-term treatment (1). The fact that peripheral resistance is elevated prior to treatment in most cases of established hypertension (4) could make it seem somewhat unlogical to institute therapy which causes a further increase of peripheral resistance.

At least from a theoretical point of view it would therefore be interesting to combine a beta receptor blocking agent with a peripheral vasodilator. This has been done both by using propranolol in combination with a new vasodilator orally (2) and by combining alprenolol and dihydralazine in acute hemodynamic studies (6).

The present report concerns the clinical effects of prolonged treatment of hypertension with a combination of propranolol and hydralazine given orally.

MATERIAL AND METHODS

Seventeen patients, who had been part of a long-term study including 167 hypertensive patients treated with large doses of propranolol, were treated with a combination of propranolol and hydralazine. A summary of the clinical information concerning these patients is presented in Table I. Of the 17 patients (10 men and 7 women, average age 50 years, range 22–72 years) 5 had renovascular hypertension and 12 essential hypertension.

The patients were seen periodically in the out-patient departments and their blood pressure was measured in the supine and standing positions at rest. Dosage of propranolol was progressively increased from 20 mg four times daily up to as much as 640 mg daily. The average daily propranolol dose was 320 mg. Hydralazine was given four times a day with an average daily dose of 137 mg and maximum daily dose of 200 mg.

Prior to the beginning of this study seven patients were treated with bethanidine or guanethidine and these drugs were withdrawn completely during the period. There were also seven patients who were taking saluretics both before and during the period of treatment; dosage was kept unchanged. Finally eight patients were on hydralazine at the start of the investigation and their dosage was either unchanged or increased.

Table I. *Clinical information*

Pat. no.	Age (y)	Sex	Fundi (K.W.B.)	Serum creatinine (mg/100 ml)	Duration of treatment (weeks)	Final dosage (mg/day) Propranolol	Hydralazine
Essential hypertension							
1	56	♀	I	0.9	32	640	200
2	63	♀	I	1.1	44	480	50
3	50	♂	II	0.9	48	320	100
4	50	♂	II	1.2	12	320	75
5	22	♀	I	1.1	10	160	100
6	55	♂	II	0.8	24	160	100
7	51	♂	I	1.0	40	320	200
8	24	♂	0	1.0	29	320	200
9	53	♂	0	1.2	48	160	100
10	56	♂	I	1.2	20	160	100
11	45	♂	II	1.2	40	320	200
12	56	♀	II	1.3	10	160	100
Renovascular hypertension							
13	43	♀	II	1.0	44	480	50
14	26	♀	I	0.6	20	160	100
15	63	♀	II	0.9	16	320	100
16	64	♂	II	1.8	20	640	200
17	54	♂	II	1.1	28	320	200
Average	50				28	320	137

RESULTS

Hypotensive effects

Arterial pressure was reduced in all 17 patients studied, although in two patients the reduction was confined to the systolic pressure. For the entire group, supine pressures fell from 201/125 mmHg to 155/100 mmHg, a difference of 46/25 mmHg ($p < 0.0005$ *t*-test). Standing pressures fell from 185/120 mmHg to 151/103 mmHg, the difference being 34/17 mmHg ($p < 0.0005$ systolic fall and $p < 0.0025$ diastolic fall). Individual changes for each patient are given in Table II. Heart rate fell from 77 to 62.

Table II. *Blood pressure measurements before and during combined therapy with propranolol and hydralazine*

Pat. no.	Initial blood pressure (mmHg) Supine	Standing	Treated blood pressure (mmHg) Supine	Standing
1	230/150	220/150	145/90	135/95
2	235/115	200/115	180/115	160/110
3	210/135	180/100	150/90	160/100
4	240/140	230/130	190/130	180/130
5	170/110	150/110	130/90	140/90
6	170/120	150/100	160/90	150/100
7	165/120	150/120	140/110	140/110
8	170/110	170/110	140/100	130/80
9	180/120	180/130	145/95	145/105
10	175/100	175/110	175/95	190/100
11	220/135	180/110	140/100	140/110
12	180/120	160/110	130/90	130/80
13	220/120	240/140	145/90	170/100
14	190/130	210/135	170/105	150/120
15	230/130	180/100	190/95	180/95
16	225/130	180/135	180/130	160/120
17	200/135	190/130	125/90	115/95
Average	201/125	185/120	155/100	151/103

These results can be compared to those obtained when propranolol was given as the only treatment in a group of 82 hypertensive patients (Fig. 1 and Table III). In the strictly propranolol-treated group there were 17 patients with renovascular hypertension and 65 with essential hypertension. The severity of their initial blood pressure elevation as well as the hypertensive changes of their fundi were not significantly different from those observed in the group treated with combined propranolol-hydralazine therapy. As seen in Fig. 1 and Table III, the reduction of arterial pressure was more pronounced in the group treated with both propranolol and hydralazine.

In the supine position the difference in arterial pressure reduction was $46/25 - 30/16 = 16/9$ mmHg ($p < 0.01$ systolic and $p < 0.025$ diastolic).

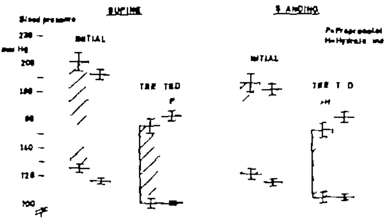


Fig. 1. Initial and treated arterial blood pressures for two groups of patients in the supine and standing positions (cf. Table III). P = propranolol, H = hydralazine.

Table III

	Supine			Standing		
	Initial	Treated	Diff	Initial	Treated	Diff.
Propranolol + hydralazine (n = 17)						
Syst.	201 ± 6.5	155 ± 5.3	46 $p < 0.0005$	185 ± 6.6	151 ± 4.9	34 $p < 0.0005$
Diast.	125 ± 3.0	100 ± 3.3	25 $p < 0.0025$	120 ± 3.7	103 ± 3.6	17 $p < 0.0025$
Propranolol (n = 12)						
Syst.	192 ± 3.3	162 ± 3.4	30 $p < 0.0005$	180 ± 3.8	160 ± 3.8	20 $p < 0.0005$
Diast.	116 ± 1.9	100 ± 1.5	16 $p < 0.0005$	114 ± 2.3	103 ± 1.9	11 $p < 0.0005$

In the standing position the difference in lowering of the blood pressure between the two groups was 34/17 − 20/11 = 14/6 mmHg ($p < 0.05$ systolic and $p < 0.10$ diastolic).

Side-effects

Major side-effects were not seen during the period of treatment. In one patient (no. 14) propranolol had to be withdrawn after five months of treatment, because the patient complained of tiredness. No signs of bronchial obstruction or cardiac decompensation were observed in any of the patients. It deserves mentioning that two patients, who were previously treated with hydralazine, reported that they felt much better after propranolol had been added to their regimen, especially noting the lack of disturbing tachycardia.

No significant changes of hematological, hepatic or renal function laboratory tests were observed during the period of treatment.

DISCUSSION

The results of this study show that a combined therapy of propranolol and hydralazine is useful as a means of lowering arterial pressure in patients with essential and renovascular hypertension. The lowering of blood pressure can be achieved with seemingly fewer side-effects than when either of the drugs is used alone. In fact no serious side-effects were seen during the period. There were no signs of postural hypotension. On the contrary the reduction of blood pressure was less pronounced in the standing position. The same observation was made in the group receiving propranolol only.

From a theoretical point of view it seems quite logical to combine propranolol and hydralazine in order to lower arterial pressure in hypertensive patients. Hydralazine, which acts mainly on precapillary vessels, causes vasodilatation and thus a reduction of peripheral resistance (8).

The main side-effects on the cardiovascular system are caused by reflexogenic sympathetic

stimulation, causing tachycardia and occasionally angina pectoris and headaches.

All these adverse effects can be effectively controlled by propranolol, which is a potent sympathetic beta receptor blocker. In fact propranolol seems to be especially well suited in this respect, as it lacks intrinsic beta receptor stimulating capability which is seen with other beta receptor blocking agents.

Propranolol is also an effective anti-hypertensive agent, both when used alone and in combination with other drugs, although the reduction of arterial pressure comes at the cost of a slight increase of peripheral resistance.

Our present policy in treating hypertension is to start with propranolol and progressively increase dosage until arterial pressure is well controlled. If reduction of blood pressure is unsatisfactory we add hydralazine to the regimen. We feel that it is possible to achieve a balance between the two drugs, so that the beneficial effects of both are combined, while the side-effects are kept at a minimum.

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COMPARATIVE STUDY OF THE CLINICAL EFFECT OF ACENOCOUMAROL (SINTROM®) AND PHENPROCOUMON (MARCOUMAR®) IN MYOCARDIAL INFARCTION AND ANGINA PECTORIS

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Abstract. A controlled clinical trial was performed in more than 250 patients with myocardial infarction or angina pectoris to determine whether any difference in clinical effect could be observed between acenocoumarol (Sintrom®) and phenprocoumon (Marcoumar®). The average observation period was almost three years. For the myocardial infarction group (both with and without relative contra-indications) there proved to be a small but significant difference in the stability of the hypocoagulability of these two coumarin preparations: phenprocoumon gave the most stable results. Judging from the mortality, morbidity and the number and severity of the bleeding complications, however, there was no statistically significant difference between the groups treated with these two anticoagulants. The favourable results obtained in this study with the short-acting acenocoumarol are based on the strict supervision of patients on prolonged anticoagulant treatment provided by the Netherlands Thrombosis Service, in which the control by nurses plays an important role.

Various studies performed since 1957 have shown that the so-called long-acting coumarins give a more stable anticoagulation than the short-acting types (2, 3, 4, 5, 9, 10, 11). However, in most of these studies the observation time was relatively brief (weeks to months) and the intensity and stability of the anticoagulant therapy inadequately reported. Furthermore, clinical repercussions of such a difference in stability have not been sufficiently studied in controlled clinical trials. These considerations led us to undertake the investigation reported here, in which the short-acting coumarin preparation acenocoumarol (Sintrom Mitis®) was compared with the longest-acting coumarin congener phenprocoumon (Marcoumar®). Included in the study were patients suffering from angina pectoris and myocardial infarction. The criteria applied were: the occurrence of myocardial reinfarction, other cardiovascular complications, and bleeding complications. The observation period of the entire study amounted to almost four years.

CLINICAL DESIGN, MATERIAL AND METHODS

Starting on 1 May 1966 at the Leiden Thrombosis Service, all newly admitted men with either angina pectoris or myocardial infarction were separately and randomly assigned to one of two groups.

One group was given acenocoumarol (1 mg tablet), the other phenprocoumon (3 mg/tablet). A total of 299 patients were included in the study: 250 with myocardial infarction and 49 with angina pectoris. Because admission of patients to our Thrombosis Service usually occurs after hospitalization, i.e. under anticoagulant treatment, many patients had to be switched from one to the other coumarin preparation. Therefore the first month of our supervision has not been taken into consideration. During this first month 31 patients had to be dropped from the study (Table I). The data for the remaining 268 patients are listed in Table II. During the observation period another 37 patients were dropped from the study (Table III). For patients without relative contra-indications we tried to obtain thrombotest times between 110 and 180 sec (about 10 and 5%, respectively); for patients with relative contraindications (older than 65 years, hypertension, status after cerebral thrombosis or cerebral bleeding and after peptic ulcer), these limits were 90 and 135 sec (about 12 and 7% respectively) (8). The patients who developed relative contra-indication during the course of the study were immediately assigned to the other group (17 patients). The reverse was done with patients whose blood pressure became normal (2 patients). This explains why the numbers shown in Tables IV, V and VI diverge from those in Table II. The frequency of coagulation control per patient was at least once every three weeks and was virtually the same for the two groups. The dosages were

Table I *Data of patients excluded during the first month of our supervision*

Reason for exclusion	Myocardial infarction group Phenprocoumon	Acenocoumarol	Angina pectoris group Phenprocoumon	Acenocoumarol
Erroneous diagnosis	3	1	1	
Change of residence	1	1		
Death	7	7		1
Psychological reasons		3		1
Absolute contra-indications	1	1		
Other reasons		2	1	
Total	1	15	2	2

set by three of the authors (J J V R. B. and N. F.). To keep the coumarin plasma-concentration within narrow limits, long-acting and short-acting preparation have to be dosed in different ways. Therefore the trial could not be double-blind.

RESULTS

The evaluation included both the thrombotest times and the clinical results, the former for the period 1 May 1966 – 1 Jan. 1969 the latter for the period 1 May 1966 – 1 Jan. 1970. All the thrombotest times obtained have been taken into consideration. The 31 patients who had to be dropped during the first month of our supervision introduced no bias (Table I). With respect to the thrombotest times, we started calculating per patient the percentage of determinations falling outside the desired ranges (%D). For the myocardial infarction groups without and with relative contra-indications, and for both anticoagulants, the distributions of the %D are shown in Table IV These distributions are subdivided into four classes according to the number of determinations per patient.

We first investigated whether there is a relationship between the %D and the number of determinations. Such a relationship, if present, would have had to be taken into consideration in the further analysis of the data. For each of the four subgroups the correlation coefficients between the two variables are low They do not have the same sign and are not significant at the 5% level. Consequently the %D for phenprocoumon and acenocoumarol may be compared without taking into account the number of determinations per patient. The same conclusion holds for the much smaller angina pectoris group.

The distribution of the %D was compared by applying Wilcoxon's two-sample test to the individual percentages per patient. Myocardial infarction patients displayed a median %D significantly higher for acenocoumarol than for phenprocoumon (Table IV). For patients with relative contra-indications T was 2.01 and p (two-sided) slightly less than 0.05 and for patients with relative contra-indications T was 2.95 and p (two-sided) approximately 0.003 Hence phenprocoumon gives a more stable hypocoagulability than acenocoumarol.

Finally the distribution of the %D was analysed with respect to the direction of the deviations. For each patient we determined the direction in which the deviations predominated. For this purpose we used the symbols > for (%H > %L), = for (%H = %L) and < for (%H < %L). For example, a patient whose total %D is 38% composed of 30% H and 8% L is scored

Table II. *Data of 268 patients included in the trial*

	Myocardial infarction group Phenprocoumon		Acenocoumarol		Angina pectoris group Phenprocoumon		Acenocoumarol	
Presence/absence of relativ contra-indications	–	+	–	+	–	+	–	+
Number	62	51	59	51	16	6	8	15
Mean age (y)	54.0	60.8	52.6	60.9	54.3	66.0	55.3	60.0

Table III. *Data of patients excluded during the observation period*

	Myocardial infarction group				Angina pectoris group			
	Phenprocoumon/ Acenocoumarol				Phenprocoumon/ Acenocoumarol			
Presence/absence of relative contra-indications	−	+	−	+	−	+	−	+
Reason for exclusion								
Change of residence	1	1	1	1	1			
Age over 65		6		5		2		
Absolute contra-indications	1	1	6	2		1		2
Other reasons		1	2[a]	1	2[b]			
Total	2	9	9	9	3	3	—	2

Including one patient excluded on the basis of excessive salivation on phenprocoumon.
Attending physician requested only 6 months of anticoagulant therapy
Including one patient with dubious coronary insufficiency

as > The results of this analysis are shown in Table V from which it is evident that in the myocardial infarction group without relative contra-indications the deviations predominate in the direction of excessively short thrombotest times for both drugs, although the phenomenon is slightly more pronounced for acenocoumarol than for phenprocoumon. For both coumarins, with increasing %D there is a tendency to an increase in the predominance of excessively short thrombotest times. On the other hand this tendency is not present in the myocardial infarction group with relative contra-indications. The corresponding data for the angina pectoris groups are shown in Table VI. Here, too, excessively short thrombotest times predominate in the angina pectoris group without relative contra-indications, and again this phenomenon is absent in the group with relative contra-indications. In the angina pectoris group without relative con-

Table IV *Relationship between the percentage deviation (%D) and the number of determinations per patient in the myocardial infarction group*

	Phenprocoumon Determinations per patient					Acenocoumarol Determinations per patient				
%D	<20	20–29	30–39	>40	Total	<20	20–29	30–39	>40	Total
Without relative contra-indications										
0–9	3	4	1	1	9	—	1	1	—	2
10–19	2	3	5	1	11	2	3	4	—	9
20–29	2	6	4	3	15	2	3	2	3	10
30–39	4	2	3	3	12	5	4	4	6	19
40–49	4	3	2	1	10	5	2	3	1	11
50–59	—	1	—	1	2	1	1	1	1	4
60–69	2	2	—	—	4	3	—	—	—	3
70–79						1	—	—	—	1
80–89						—	1	—	—	1
Total	17	21	15	10	63	19	15	15	11	60
Median (%)	30	25	23	28	25	42	21	31	33	33
	= −0.08 $p > 0.50$					= −0.21 $p > 0.05$				
With relative contra-indications										
0–9	1	1	—	—	2					
10–19	3	2	1	—	6	1	1	—	—	2
20–29	1	6	2	2	11	2	3	1	—	6
30–39	2	2	8	2	14	3	6	2	3	14
40–49	6	7	4	4	21	2	4	5	1	12
50–59	1	—	1	—	2	4	4	3	4	15
60–69	1	1	—	—	2	1	1	—	1	3
70–79	—	2	—	—	2	2	1	—	—	3
80–89						1	—	—	—	1
Total	15	21	16	8	60	16	20	11	9	56
Median (%)	41	36	35	41.5	38.5	49.5	38	48	50	45.5
	= −0.02, $p > 0.75$					= −0.02, p 0.75				

Table V *Trends of divergent thrombotest times in the myocardial infarction group*

The percentage of thrombotest times deviating from the range aimed at is the %D. Under = is listed the number of patients for whom the deviations are equally distributed. For the group without relative contra-indications, under > is listed the number of patients in whom deviations over 180 sec, under < those in whom deviations under 110 sec exceeded the number of deviations in the opposite direction. In the group with relative contraindications, under > is listed the number of patients in whom deviations over 135 sec, under < those in whom deviations under 90 sec exceeded the number of deviations in the opposite direction.

	Phenprocoumon				Acenocoumarol			
D	>	=	<	Total	>	=	<	Total
Without relative contra-indications								
0–9	3	1	5	9	1	—	1	2
10–19	4	1	6	11	1	1	7	9
20–29	6	—	9	15	1	2	7	10
30–39	3	1	8	12	2	—	17	19
40–49	3	—	7	10	2	—	9	11
50–59	—	—	2	2	—	—	4	4
60–69	—	—	4	4	—	—	3	3
70–79					—	—	1	1
80–89					—	—	1	1
Total	19	3	41	63	7	3	50	60
With relative contra-indications								
0–9	1	—	1	2				
10–19	3	—	3	6	—	—	2	2
20–29	5	1	5	11	1	2	3	6
30–39	8	1	5	14	6	—	8	14
40–49	10	2	9	21	5	—	7	12
50–59	1	—	1	2	5	2	8	15
60–69	1	—	1	2	2	—	1	3
70–79	1	—	1	2	2	—	1	3
80–89					—	—	1	1
Total	30	4	26	60	21	4	31	56

tra-indications the %D shows no difference between acenocoumarol and phenprocoumon, for the angina pectoris group with relative contra-indications the %D found for the phenprocoumon group seems, on the average, to lie even higher than for the acenocoumarol group (median $M = 50$ and $S = 34$ two-sided application of Wilcoxon's two-sample test, p just under 5%).

The clinical evaluation included both total mortality and morbidity subdivided according to (recurrent) myocardial infarctions and other cardiovascular complications (Table VII) and bleeding complications (Table VIII). The numbers of patients within these subgroups do not differ significantly. Hence the two drugs do not differ with respect to the clinical results.

DISCUSSION

In the present study we tried to find out whether there is any appreciable difference in therapeutic effect between the longest-acting coumarin (phenprocoumon) and a short acting coumarin (in this case acenocoumarol). As case material we chose patients suffering from coronary atherothrombosis. The control method used for measuring the hypocoagulability was the thrombotest, since 1964 adopted by the Netherlands Thrombosis Service. The criteria used for the clinical evaluation were the occurrence of cardiovascular (i.e. thrombotic) complications and bleeding complications. These clinical parameters could be considered relevant because of the favourable results obtained in a double-blind trial with phenprocoumon versus placebo, performed under similar conditions (8). The results in the myocardial infarction groups indicate that the stability of the hypocoagulability achieved with phenprocoumon is higher than that obtained with acenocoumarol. The difference

Table VI. *Trends of divergent thrombotest times in angina pectoris patients*

For explanation see legend to Table V

	Phenprocoumon				Acenocoumarol			
%D	>	=	<	Total	>	=	<	Total
Without relative contra-indications								
0–9	—	—	1	1				
10–19	1	—	1	2				
20–29	1	—	2	3	—	1	1	2
30–39	—	—	5	5	—	—	4	4
40–49	1	—	1	2				
50–59	—	—	1	1				
60–69	—	—	1	1	—	—	1	1
70–79					—	—	1	1
80–89	—	—	1	1				
Total	3		13	16	0	1	7	8
Median (%)				37.5				35
With relative contra-indications								
0–9					—	—	1	1
10–19								
20–29					2	—	2	4
30–39					2	2	2	6
40–49	3	—	—	3	—	—	1	1
50–59	1	1	1	3	—	1	3	4
60–69								
70–79	1	—	—	1				
80–89					1	—	—	1
Total	5	1	1	7	5	3	9	17
Median (%)				50				34

is not great when the medians of the %D per patient are taken as parameter which may be done because there is no significant difference between the %D and the number of determinations per patient (Table IV). In the angina pectoris group with relative contra-indications, the median of the %D found for patients treated with phenprocoumon lies even higher than for patients treated with acenocoumarol (Table VI) but because of the limited number of patients in this group this difference cannot be considered significant.

Considering the myocardial infarction group without relative contra-indications, it is interesting to see that the less stable the hypocoagulability the shorter the thrombotest times tend to be (Table V) This is hardly surprising, however since unstable coagulation is often the result of irregular intake of the prescribed daily dose by the patient.

The remarkable similarity in stability obtained with the short and long-acting coumarin preparations must, in our opinion, be ascribed to the strict guidance of the patients maintained by the Thrombosis Service nurses (7) who in addition to other factors pay particular attention to the regular intake of the prescribed dose. In view of the relatively small pharmacodynamic difference found between acenocoumarol and phenprocoumon, it is not surprising that no difference appeared in the clinical effect either with respect to the mortality and cardiovascular morbidity (Table VII) or with respect to the bleeding complications observed (Table VIII) The observation time, which averaged almost three years, may be considered sufficiently long to justify the conclusion that from a therapeutic point of view the two preparations are equally effecti e, but on the condition of experienced dosing and strictly supervised patients.

Table VII. *Occurrence of deaths (cardiac and non-cardiac), myocardial (re)infarctions, and other cardiovascular complications during the study*

	Myocardial infarction group				Angina pectoris group			
	Phenprocoumon/		Acenocoumarol		Phenprocoumon/		Acenocoumarol	
Presence/absence of relative contra-indications	–	+	–	+	–	+	–	+
Non-cardiac deaths		3	2	1				
Sudden cardiac deaths	1	1		3	1			
(Re)infarctions	3[a]	2[b]	4	4	1			1
Other cardiovascular complications		1	1	1	1		1[f]	

All three died in the acute phase of recurrent myocardial infarction.
One of these patients died two months later cause unknown.
Died in state of asthma cardiale.
Died due to cardiac insufficiency organized myocardial infarction observed post mortem.
Phlebitis.
[f] Thrombosis of deep calf ein.

Table VIII. *Number and localization of bleeding complications during the study*

	Myocardial infarction group				Angina pectoris group			
	Phenprocoumon/		Acenocoumarol		Phenprocoumon-		Acenocoumarol	
Presence, absence of relative contra indications	–	+	–	–	–	+	–	
Skin	4			1	1			
Respiratory tract	4[a]	3	2		1			1
Digest. tract	1		2	3[d]				
Urogenit. tract			6			2	1[f]	1
Eyes	2[h]		1		1			
Central nerve system		1		1[j]	1			1[k]

Of whom three ith recurrent epistaxis
One of whom had epistaxis due to nasal polyps.
Showed simultaneously also short-lasting haematuria.
Including one patient with recurrent haemorrhoidal loss of blood.
Two of hom had recurrent haematuria.
[f] Also showed haematoma in the upper leg.
Also displayed epistaxis.
One of hom had recurrent conjunctival bleedings, the other multiple haemorrhages of the fundus.
[i] Recurrent conjunctival bleeding, also haematoma in leg and gastric bleeding.
[j] Intracerebral haematoma ery probable, termination of anticoagulant therapy further course unknown.
Died, cerebral haemorrhage demonstrated post mortem.

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RENAL OXYGEN CONSUMPTION AND MEDULLARY OXYGEN TENSION IN CHRONIC HYPOXIA IN MAN

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Abstract. This study was undertaken to elucidate the factors responsible for the renal lesion in congenital cyanotic heart diseases. Four patients with septal defects and four with Fallot's tetrad and renal involvement have been examined by right heart catheterization and reexamined by renal vein and kidney pelvic catheterization for determination of the renal functional and metabolic impairment simultaneously with oxygen tension measurement in pelvic urine. The renal involvement in these patients was not correlated to the degree of polycythemia. Pressure measurements in the right atrium contradict the hypothesis that the renal involvement is attributed to increased pressures on the right side of the heart. Studies of the renal oxygen extraction and renal oxygen consumption simultaneously with oxygen tension measurements in pelvic urine do not support the assumption that the renal lesion is due to the hypoxia per se. We assume that hemodynamic changes are the primary factors inducing the renal involvement in patients with congenital cyanotic heart diseases.

Proteinuria and progressive renal insufficiency can be found in patients with congenital cyanotic heart diseases. Characteristic glomerular changes have been observed in some of these patients. The pathogenesis of the renal involvement is unknown. The aim of the present study has been to examine renal functional and metabolic changes in this state of chronic hypoxia for the purpose of elucidating the pathogenesis of the renal lesion in congenital cyanotic heart disease.

MATERIAL AND METHODS

Eight patients with cyanotic heart diseases have been studied. Age, sex and diagnosis are presented in Table I. All patients were studied by right heart catheterization. Table II presents the systemic cardiac output and the right-to-left shunt in percentage of systemic cardiac output, together with the mean pressure in the right atrium. This pressure is normal in all patients, except in cases 6 and 7, in whom the pressure is slightly increased.

The patients were reexamined by catheterization of the right renal vein simultaneously with retrograde catheterization of the right renal pelvis, except patient 8. Blood samples were drawn from the femoral artery and right renal vein for determination of oxygen tension and saturation. Blood oxygen saturation and oxygen tension measurements in blood and in pelvic urine were performed in accordance with methods previously described (3).

RESULTS

The arterial oxygen saturation (ApO_2 sat.) was decreased below 80% in four patients, in one below 70% (Table III). The arterio-venous oxygen difference (AV diff.) (oxygen extraction) varied from 0.90 to 2.55 Vol.%, average 1.68 Vol.%, which is in the normal range. Urine from the right renal pelvis was collected simultaneously with the blood sampling. Mean value for UpO_2 was 34 (range 47–27). Despite a significant decrease in ApO_2 (mean [illegible] mmHg), UpO_2 was slightly reduced. UpO_2 was in the normal range for all patients. The mean gradient ($RVpO_2 - UpO_2$) was 8.3 mmHg, which is significantly below the gradient in normal individuals. Renal function studies were performed simultaneously with the oxygen determination. Glomerular filtration rate was measured as inulin clearance, and renal plasma flow as clearance of PAH/PAH extraction rate. Inulin was determined by the method of Schreiner (21) and PAH by the method of Finkelstein et al. (7). The results of these studies are presented in Table IV, while the results of renal oxygen consumption determination are presented in Table III.

Renal oxygen consumption varied according to change in renal blood flow, except in cases 4 and 8, in whom oxygen consumption was decreased also due to a low oxygen extraction.

Table I. *Age, sex and diagnosis of the patients studied*

Pat. no.	Age (y.)	Sex	Diagnosis
1	44	♂	Atrial septal defect
2	54	♂	Atrial septal defect
3	19	♂	Tetralogy of Fallot
4	21		Tetralogy of Fallot
5	4	♂	Atrial and ventricular septal defect
6	21	♀	Ventricular septal defect Pulmonary stenosis
7	30	♀	Tetralogy of Fallot
8	48	♂	Tetralogy of Fallot

Table II. *Systemic cardiac output (C.O.), right-to-left shunt and atrial pressure in the patients studied*

Pat. no.	C.O. (l/min)	Right-to-left shunt (% of C.O.)	Right atrial BP (mmHg) Systolic	Diastolic	Mean
1	8.8	63	9	4	5
2	4.7	15	5	−3	0
3	3.4	34	6	1	4
4	6.5	56	6	1.5	4
5	5.5	71	4	1.5	2.5
6	5.4	16	13–10	6–4	8
7	8.0	76	15–12	2	10
8	4.7	6	12	1	5

DISCUSSION

The renal involvement in patients with congenital cyanotic heart diseases is not due to thromboembolic processes in the kidney or to bacterial endocarditis with focal embolic glomerulonephritis (25, 26). Some of the glomerular changes can be seen in connection with other renal diseases (25, 26). These renal diseases have not been present in our patients.

Glomerular involvement, proteinuria and renal insufficiency are inconstant findings in congenital cyanotic heart diseases, and the pathogenesis of the renal lesion is unknown. A number of theories have been postulated. Meeson and Litton (16) assumed that chronic hypoxia and increased carbonic dioxide content in the blood are the direct causes of the glomerular changes. Marinozi (14) attributed the glomerular changes to the secondary polycythemia and not to hypoxia per se. He believed that the glomerular enlargement which is observed in congenital cyanotic heart diseases was due to a numerical increase in capillary loops as a compensatory result of the decrease in renal plasma flow. Increase in capillary surface available for ultrafiltration in the glomeruli would compensate for the decrease in plasma perfusing the glomeruli. Imai (11) believed that the glomerular changes were effected by a complex of pathogenetic factors including polycythemia, ischemia and congestion which were ultimately related to the degree of hypoxia. This is not in accordance with the opinion of Spear (24, 25) who could not demonstrate any correlation between the degree of glomerular changes and polycythemia or degree of cyanosis. He claimed that similar glomerular changes have been demonstrated both in primary pulmonary hypertension and congenital cyanotic heart diseases without polycythemia. Spear believed that increased blood pressure on the right side of the heart might be an important etiologic factor and he actually observed glomerular changes in patients with cor pulmonale. These observations were later confirmed by Ellis (5) and Caldwell et al. (4). As a hypothetical mechanism Spear argued that increased renal vein pressure is likely to occur in right heart failure and could be responsible for the glomerular changes in anu

Table III. *Blood oxygen saturation and oxygen tension measurements*

Pat. no.	Arterial PO_2 (mmHg)	Arterial Sat. ( )	Renal PO (mmHg)	Venous Sat. ( )	A-V-diff (Vol.)	Urine PO (mmHg)	Renal oxygen cons. (ml/min)
1	39	64	35	53	1.09	27	7.1
2	57	88	44	78	1.61	47	6.6
3	47	78	37	66	2.55	37	22.4
4	47	80	44	77	0.90	28	8.6
5	43	79	36	71	1.87	31	10.8
6	52	85	43	77	1.87	36	16.3
7	44	77	38	70	1.92	33	11.7
8	59	89	52	86	0.82	—	5.4

Table IV *Results of renal function studies*

Pat. no.	GFR (C_{In}) (ml/min)	E_{PAH} (%)	RPF (ml/min)	Hct. (Vol. %)	RBF (ml/min)	RBF/C.O. (%)	FF
1	58	87	331	40	651	7	0.18
2	69	87	290	35	446	10	0.24
3	83	49	447	46	878	26	0.19
4	81	86	333	65	951	15	0.24
5	64	87	264	54	574	11	0.24
6	83	85	363	51	740	14	0 23
7	61	85	220	64	608	8	0.28
8	76	93	321	51	654	14	0.24

logy with proteinuria observed in congesti e heart failure, pericarditis and renal vein thrombosis. Our pressure measurements in the right atrium (Table II) contradict the opinion of Spear In 6 of our 8 patients examined, the right atrial pressure was normal. Only in patients 6 and 7 was this pressure slightly increased.

As will be seen from Table IV there is no correlation between the functional impairment of the kidney and the degree of polycythemia. Our findings in this respect do not support the hypothesis that the renal involvement is correlated to the secondary polycythemia. Renal hemodynamic and metabolic studies during systemic hypoxia have been conflicting. The divergent results are partly due to differences in the duration of the hypoxia, partly to differences in the degree of hypoxia. During acute hypoxia a decrease in renal blood flow and in the renal fraction of car diac output has been found (2, 4 8, 9 10 15 19 22, 23), while an increase in renal vascular resistance takes place (6, 9 10). Simultaneously a decrease in GFR has been reported (6, 9 10, 19 26, 28), but to a lesser extent than the fall in renal blood flow Consequently the filtration frac tion (FF) will increase. AV oxygen difference has been found to be unaltered by most investigators (2, 6, 9 10, 26, 27) Renal oxygen consumption will consequently decrease in relation to the decrease in renal blood flow

Renal hemodynamic and metabolic studies in chronic hypoxia are few Recently Engelman et al. (6) examined dogs after experimental chronic hypoxia. They found increased renal blood flow and a decrease in renal vascular resistance. The oxygen consumption did not differ significantly from the normal, indicating a decrease in oxygen extraction. This decrease in oxygen extraction is not significant but has the result that oxygen consumption is not correlated to the changes in renal blood flow The degree of hypoxia in this study was relatively mild. Thurau found a de crease of oxygen consumption during hypoxia of more severe degree (average arterial oxygen sa turation 35%) (26, 27) The reduction of oxygen consumption was due to a decrease in oxygen extraction, since RBF in his study was unchanged.

In our study the AV oxygen difference was in the normal range except in two cases. The renal oxygen consumption varied, with concomitant changes in renal blood flow Simultaneously the UpO_2 reflecting medullary oxygen tension was in the normal range. These findings indicate that the oxygen supply to the renal tissue was ade quate and that the renal lesion in these cases was not due to the systemic hypoxia per se

We believe that hemodynamic changes might be the important factor in this respect. Renal blood flow was significantly reduced in all cases, in contrast to other studies. The renal fraction of cardiac output was significantly reduced in all cases except one (patient 3) FF was in the nor mal range for all patients except one (patient 7) indicating normal renal vascular resistance in these patients.

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1	8.8	63	9	4	5
2	4.7	15	5	−3	0
3	3.4	34	6	1	4
4	6.5	56	6	1.5	4
5	5.5	71	4	1.5	2.5
6	5.4	16	13–10	6–4	8
7	8.0	76	15–12	2	10
8	4.7	26	12	1	5

DISCUSSION

The renal involvement in patients with congenital cyanotic heart diseases is not due to thromboembolic processes in the kidney or to bacterial endocarditis with focal embolic glomerulonephritis (25, 26). Some of the glomerular changes can be seen in connection with other renal diseases (25, 26). These renal diseases have not been present in our patients.

Glomerular involvement, proteinuria and renal insufficiency are inconstant findings in congenital cyanotic heart diseases, and the pathogenesis of the renal lesion is unknown. A number of theories have been postulated. Meeson and Litton (16) assumed that chronic hypoxia and increased carbonic dioxide content in the blood are the direct causes of the glomerular changes. Marinozi (14) attributed the glomerular changes to the secondary polycythemia and not to hypoxia per se. He believed that the glomerular enlargement which is observed in congenital cyanotic heart diseases was due to a numerical increase in capillary loops as a compensatory result of the decrease in renal plasma flow. Increase in capillary surface available for ultrafiltration in the glomeruli would compensate for the decrease in plasma perfusing the glomeruli. Imai (11) believed that the glomerular changes were effected by a complex of pathogenetic factors including polycythemia, ischemia and congestion which were ultimately related to the degree of hypoxia. This is not in accordance with the opinion of Spear (24, 25) who could not demonstrate any correlation between the degree of glomerular changes and polycythemia or degree of cyanosis. He claimed that similar glomerular changes have been demonstrated both in primary pulmonary hypertension and congenital cyanotic heart diseases without polycythemia. Spear believed that increased blood pressure on the right side of the heart might be an important etiologic factor and he actually observed glomerular changes in patients with cor pulmonale. These observations were later confirmed by Ellis (5) and Caldwell et al. (4). As a hypothetical mechanism Spear argued that increased renal vein pressure is likely to occur in right heart failure and could be responsible for the glomerular changes in ana-

Table III. *Blood oxygen saturation and oxygen tension measurements*

Pat. no.	Arterial PO_2 (mmHg)	Arterial Sat ( )	Renal PO (mmHg)	Venous Sat ( )	A-V-diff (Vol. %)	Urine PO (mmHg)	Renal oxygen cons. (ml/min)
1	39	66	35	55	1.09	27	7.1
2	57	88	44	78	1.61	47	6.6
3	47	78	37	66	2.35	37	22.4
4	47	80	44	77	0.90	28	8.6
5	43	79	36	71	1.87	31	10.8
6	52	85	43	77	1.87	36	16.3
7	44	77	38	70	1.92	33	11.7
8	59	89	52	86	0.82	—	5.4

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INTERMITTENT BENIGN RENAL HEMATURIA

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Abstract. Thirty-one patients with focal glomerular changes in the renal biopsy are reported. Focal glomerular lesions have been seen in the following diseases: benign recurrent hematuria (19 patients), systemic lupus erythematosus (7 patients), nephrotic syndrome (2 patients), Henoch-Schönlein purpura (1 patient), sarcoidosis (1 patient), systemic disease with myocarditis (1 patient). Focal glomerulonephritis is a pathological diagnosis and should be avoided in clinical classification. To retain this term in the histologic classification of renal diseases is in our opinion debatable, due to the fact that the term might convey the impression that focal glomerulonephritis is a specific disease entity. To discuss common etiological factors for this group of diseases seems irrelevant. The task for the pathologist is to demonstrate differential findings in the biopsies that might be of value for the clinical differentiation of the diseases that can show focal glomerular changes on light microscopy of the renal biopsy.

The development of renal biopsy for direct examination of the renal lesion has provided a valuable aid in the diagnosis of renal diseases. In attempts to classify renal diseases the nephrologist, however, due to a limited diagnostic armamentarium, may overemphasize the significance of the histologic changes in the renal biopsy. One example in this respect is focal glomerulonephritis, a term also applied in clinical classification. Focal glomerulonephritis is a histologic diagnosis and has no distinct clinical counterpart. It has been clearly demonstrated that focal glomerular lesions can be found in patients with benign recurrent hematuria (1, 2, 3, 4, 5, 9, 12, 13), polyarteritis nodosa (5, 8), systemic lupus erythematosus (5, 7, 8), Henoch-Schönlein's syndrome (5, 7, 8), pulmonary hemorrhage and glomerulonephritis (Goodpasture syndrome) (5, 7, 8), focal embolic nephritis (the renal lesion commonly seen in subacute bacterial endocarditis) (7, 8) and in special cases with hereditary nephritis (8). Focal glomerular lesions may probably be demonstrated in connection with other renal diseases. The clinical use of the term focal glomerulonephritis is therefore irrelevant. Attempts to describe focal glomerulonephritis as an entity or to discuss common etiological factors in this heterogeneous group of renal diseases that might show similar glomerular changes on light microscopy of the renal biopsy may produce confusion. The pathogenesis of the focal glomerular lesion may however be common.

The task in this respect is to demonstrate possible differential findings in the renal biopsy from cases with focal glomerular lesion that might be of significance for the clinical diagnosis. The studies of Berger et al. (4) and Munthe et al. (10) are steps in the direction of differentiating the focal glomerular changes. These authors have made an attempt to differentiate this group of renal diseases by immunofluoroscopic examination of the renal biopsy. Still more data are needed regarding the renal diseases showing focal glomerular changes. Clues for the clinical diagnosis are necessary, but longitudinal studies of renal biopsies in cases with focal glomerular changes are also important in providing evidence for the development of these renal diseases.

MATERIAL AND METHODS

Of about 300 renal biopsies performed in our department in the last years glomerular lesion was focal in nature in 31 cases. Our renal biopsies have been taken during X-ray examination with TV equipment as previously described (6). Only biopsies with more than 10 glomeruli have been regarded as representative. The clinical data are given in Table I. As will be seen, the largest group is benign recurrent hematuria, comprising 19 patients. The glomerular lesion was focal in 7 patients with systemic lupus erythematosus. Two patients had nephrotic syndrome. In one patient with Henoch-Schönlein's syndrome

Table I. *Clinical data*

Pat. no.	Sex	Age at onset (y)	Duration of disease (y)	Proteinuria (‰)	Epit. casts	Hematuria Macroscopic	Hematuria Microscopic	Provoking factors	Intermittent hematuria	Serum creat. (mg/100 ml)	BP (mmHg)	ASO	LE factor tests
1		4	9	–	–	+		Throat infection		0.6	115,60	160	–
2	♀	7	3	Traces	(+)	+		Throat infection	+	0 6	110/70	800	–
3	♂	9	2	–	–	–		N	–	0.6	120/70	200	–
4	♂	11		T aces	( )	+		Throat infection	–	0.7	130,80	120	–
5	♂	13	4		–	+		Throat infection		0.7	110 60	25	–
6	♂	14	5	–	( )	+		No	+	1 1	100/70	200	–
7		20	4		–			No	–	0.9	120/80	60	–
8		23	3	5	(+)	+		No	–	0.8	130,80	500	–
9		23	4	Traces	–			Catarrhal	–	0.7	160,85	160	–
10	♂	23		Traces	–	+		Fever	–	0.9	175,80	250	
11	♂	4	4		–		+	No	+	1 4	125,85	160	
12		41	3		–		+	No	–	0.9	125/70	320	–
13	[illegible]	59	4	–	( )		+	No	–	1.0	125/75	100	–
14		22	3				+	No		0 8	150/100	25	–
15	♂	6	1		–			Fever		0 5	95/75	50	–
16	♂	22	1		–			Fever	–	1.2	150,90	40	–
17		13	4	Traces			+			0.8	180,80	960	
18		11	15	2			–			0.7	135,80	25	( )
19		11	5			–	–	Throat infection		0.7	115/75	25	– ( )
20		26	8		–			Fever		1.0	115/75	60	
21		31	2	2	( )			Fever		1 4	150,90	30	
22		39	8	4				Fever		1.2	170,100	50	
23		39		T aces	( )			Fever		0.9	130,85	660	
24	♂	48	1	12			+	Fever		1 2	130 80		( )
25		45	1				( )			1.4	[illegible]20/150	100	
26		15	1				–			0.7	130 85	250	
27		47	1	8			–			1 0	140 100	320	
28	♂	9	8	7			–	Fever		0.8	120,80	120	
29	♂	5		12						0 7	100/70	[illegible]5	
30	♂	53	4				–			1 4	110 80	50	
31		5		Traces			–		–	2 3	130 80	200	

ith renal involvement the glomerular lesion as focal. One patient ith myocarditis had focal glomerular lesion ith proteinuria. One patient with sarcoidosis had also renal disease ith proteinuria and decreased renal function. Examination of the renal biopsy revealed focal glomerular changes.

CLINICAL AND PATHOLOGICAL FINDINGS

Benign recurrent hematuria

The age of the patients in this group aried from 4 to 59 years hen the hematuria started. About 50% of the patients ere below the age of 15 years. Episodes of hematuria had lasted to 9 years, in most of the patients between 2 and 5 y ars. The enal function as normal and the BP within the normal range in all patients. Eleven of the 19 patients had proteinuria, but as ill be seen from Table I, proteinuria as negligible in most cases. In four patients microscopic examination of the urine revealed epithelial casts. Edema had not been present in these patients. ESR as normal in all patients, but two had elevated ASO titer. The episodes of hematuria ere preceded by throat infection in only four patients. The hematuria started in one patient after ffort. In the other patients no factor provoking the hematuria could be demonstrated.

On intravenous pyelography and cystoscopic examination in all patients no lesion that might cause the hematuria was discovered. Histologic examination of the renal biopsy revealed focal and local glomerular changes. F cal glomerular lesion is characterized by changes in only some f the glomeruli, hile the rest are normal. Only one or tw lobules might be affected, while the rest of the glomerulus is normal as seen by light microscopy. In few cases generalized changes in the affected g omeruli hav been seen. Usually the changes are located peripherally in the glomeruli and ar of the prolif ative type. Fibrous or hyaline changes with adhesion to Bow

... capsule ... ere frequently found. In none of the cases *in this group have local necroses been observed.*

Proliferative changes may be found in some glomeruli, while the predominant changes in other glomeruli in the same biopsy show fibrosis or hyaline changes. In some patients rebiopsy was performed after 1 2 years. We have tried to compare the changes in the second and, in one case, third biopsy with the findings in the first biopsy. In some patients significant increase in the fibrotic or hyaline changes was found in the second or third biopsy. In other patients no such difference could be demonstrated. But, as will be seen from Table I, the disease had lasted for some years in most of the cases and we have therefore had no opportunity to examine the patient in the initial phase. Tubular, vascular and interstitial changes were minimal.

Systemic lupus erythematosus

The patients in this group were aged from 15 to 48 years. The disease had lasted for 1 to 8 years before the biopsy was taken. All had verified SLE with circulating nuclear antibodies and LE-cells in the peripheral blood. Proteinuria was present in all patients. In two patients nephrotic syndrome was diagnosed. Microscopic hematuria was present in all patients. BP was elevated in two patients and the renal function slightly reduced in one. Light microscopic examination of the kidney biopsy revealed focal necrotic glomerular changes in addition to proliferative changes. In some biopsies hematoxylin bodies were seen. Localized thickening of the basement membrane also in the form of wire-loop changes in the area with necrosis and proliferative changes has usually been found. Intracapillary hyaline thrombi have rarely been observed. The tubular changes have been minimal, but often interstitial infiltration with chronic inflammatory cells has been observed.

Myocarditis with focal glomerular changes

A 58-year-old man who previously had been in good health got viral infection in 1965. Since 1965 he had had paroxysmal atrial fibrillation, and later progressive cardiac insufficiency developed. Since Sept. 1969 inconstant proteinuria but normal renal function was found. ESR was normal. Circulating nuclear antibodies could not be demonstrated and LE-cells were not found in peripheral blood. Renal biopsy showed mostly normal glomeruli, but, in some, proliferative changes were found localized peripherally in the glomeruli. In the same area basal membrane thickening was observed. No vascular or tubular changes could be demonstrated. Small interstitial areas with infiltration of chronic inflammatory cells were seen.

Sarcoidosis

A 52-year-old female who in Jan. 1970 had short-lasting episode of fever developed, one month later fleeting joint symptoms and skin eruption diagnosed as erythema nodosum. ESR rose to 100 mm, ASO was normal. Urine examination revealed proteinuria and microscopic examination showed both hyaline and epithelial casts. There was no hematuria. Creatinine clearance was decreased to 36 ml/min and serum creatinine was 2.3 mg/100 ml. Circulating nuclear antibodies could not be demonstrated, nor LE-cells in peripheral blood. Quantitative analysis of immunoglobulins revealed increased IgG fraction. Serum calcium level was normal.

On light microscopy of the renal biopsy typical focal glomerular lesions were found with local proliferative changes and basal membrane thickening in the same area. No vascular changes could be demonstrated and the tubules were normal. The interstitial changes were minimal. In small areas infiltration with chronic inflammatory cells and slight fibrosis were found. No sarcoid tissue could be demonstrated in the renal biopsy. The diagnosis sarcoidosis was verified by microscopic examination of a lymph node.

The two cases with nephrotic syndrome revealed no specific feature of interest in this respect.

DISCUSSION

When focal and local glomerular changes are found in renal biopsies, systemic lupus erythematosus, Henoch-Schönlein's purpura, Alport syndrome and polyangitis can easily be separated on the basis of the clinical findings. The group which is left will mainly consist of patients with benign intermittent hematuria. About 50% of our patients with focal glomerular lesion had benign intermittent hematuria. Two of 31 patients had nephrotic syndrome. One patient had a systemic disease with myocarditis and renal involvement. In one patient with sarcoidosis focal glomerular lesions were found. No specific granulomatous sarcoid tissue could be demonstrated in the renal biopsy.

Groups of diseases showing focal glomerular lesion on light microscopy will probably increase, *indicating that the term focal glomerulonephritis* has no significance in histologic classification. The histologic term is probably only of interest in cases with benign intermittent hematuria. For many years Volhard's (14) clinical description of focal glomerulonephritis was accepted. In 1940 Payne and Illingworth (11) reported an analysis of 301 cases with acute nephritis and 64 seen in the subsequent phases. They analysed this group of patients on the basis of Volhard's assumption that edema did not occur in focal glomerulonephritis *with benign intermittent hematuria.* They divided the cases into the three following groups: one without edema, one without edema other than puffiness of the eyelids, and one with definite edema. No difference was found between these groups regarding etiologic factors, causative infection, symptomatology, degree of renal

insufficiency, hypertension or oliguria. The prognosis in the three groups was equally serious. The authors concluded on the basis of this retrospective analysis that acute focal glomerulonephritis is not a clinical entity. Their analysis did not allow this conclusion, although later studies have clearly demonstrated that focal glomerulonephritis should be avoided as a clinical term. Benign recurrent hematuria as described by Volhard is probably a special, mild form of glomerulonephritis with little or no tendency to progression. Longitudinal biopsy studies (8) indicate that there is a tendency to healing also after long-standing active disease with episodes of hematuria. In our repeated biopsies the proliferative changes were not as prominent as in the first biopsies. Hyaline glomerular changes, on the contrary, dominated in later biopsies. Benign recurrent hematuria is often misinterpreted as chronic diffuse glomerulonephritis and the serious prognosis of the disease is often exaggerated. Renal biopsy is therefore of special importance in recurrent hematuria. This disease is most frequently seen in children, but may be found in adults. The etiology is unknown, but an infectious origin is most likely.

Treatment with steroids (15, 16) and azathioprine (15, 16) has been recommended. The renal lesion is benign and such treatment seems hazardous. In other diseases (SLE, Goodpasture syndrome) this treatment might be of value (8).

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THE EFFECTS OF MORPHINE ON THE ARTERIAL AND MIXED VENOUS BLOOD GAS STATE AND ON THE HEMODYNAMICS IN PATIENTS WITH CLINICAL PULMONARY CONGESTION

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Abstract. The arterial and mixed venous blood gases and the pressures in the systemic and pulmonary circulation have been determined before and after an injection of 1.5 cg of morphine chloride intramuscularly in eight patients with left heart failure and auscultatory and roentgenologic signs of pulmonary congestion. Before morphine all patients showed clearly elevated mean pulmonary artery pressure (PAP), while only two of them showed markedly increased mean pulmonary capillary venous pressure (PCVP). All patients showed normal $PaCO_2$, low PaO_2 and low SaO_2, except the two patients with high PCVP who showed markedly reduced $PaCO_2$ and normal PaO_2. All patients showed clearly reduced $P\bar{v}O_2$ and $S\bar{v}O_2$, and an abnormally high $a-\bar{v}\,O_2$ difference, suggesting poor myocardial function or cardiac output response to hypoxia. The base excess was, however, slightly increased, indicating that there was no tendency to metabolic acidosis. After morphine the two patients with increased PCVP showed a clear fall in mean PAP, mean systemic arterial pressure (FAP) and heart rate, while only one of them showed a transient fall in PCVP. In the other patients there was no change in any of these parameters. There was a slight increase in $PaCO_2$, reaching maximum about 60 min after the injection, and a relatively smaller decrease in PaO_2, indicating some respiratory depression, but no further impairment of the alveolar/arterial gas exchange. $P\bar{v}O_2$ and $S\bar{v}O_2$ remained unchanged and $a-\bar{v}\,O_2$ was thus reduced. The base excess remained unchanged. The constancy of the base excess and $S\bar{v}O_2$ showed that no impairment of tissue oxygenation occurred after morphine, not even in the two patients with high PCVP and a marked fall in FAP after morphine. The data suggest that morphine led to a reduction of the cardiac work in the two patients with high PCVP. The remarkable constancy of $P\bar{v}O_2$ throughout the investigation, even in the presence of substantial fluctuations in PaO_2, SaO_2 and systemic blood pressure, suggests some O_2-dependent regulation of the peripheral circulation on the venous side.

Although morphine has been widely used in the treatment of patients with cardio-circulatory failure for many years, it is only recently that the influence of morphine on the cardiac and respiratory function in such patients has been more thoroughly studied. However, relevant investigations are still few and have not given a uniform picture of the consequences of morphine administration. Some authors have warned against the use of morphine in patients with myocardial infarction because of its tendency to induce hypotension, respiratory depression and ventilation/perfusion disturbances (4, 5, 11). Others, studying other types of cardiac disease, claim that morphine may safely be given, even in large doses (6).

Previous studies in this hospital have shown that the injection of 1 cg of morphine chloride intramuscularly usually has no adverse effects on the blood pressure, cardiac output or alveolar gas exchange in patients with uncomplicated acute myocardial infarction (2, 3). However, in a few patients with acute myocardial infarction and clinical signs of pulmonary congestion morphine led to a definite reduction in alveolar ventilation and a consequent further fall in arterial oxygen values (3). Since morphine is commonly used in patients with pulmonary congestion there is a need for further studies of the effects of morphine in such patients. This article presents observations on the effects of intramuscular injection of 1.5 cg morphine chloride on the arterial and mixed venous blood gas state and on the hemodynamics in patients with pulmonary congestion.

MATERIAL

Eight patients, three women and five men, aged 57 to 78 years, with clinical pulmonary congestion were studied.

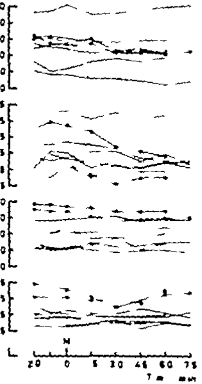


Fig. 1. The effect of 1.5 cg morphine chloride i.m. on heart rate (*HR*), mean FAP, mean PAP and mean PCVP in eight patients with clinical pulmonary congestion. *M* indicates injection of morphine. The curves marked by ○ (patient A) and ● (patient B) represent the patients with the most marked elevation of PCVP and largest fluctuations in the arterial blood gas state.

The diagnosis was based on the findings of basal crepitations over the lungs and on roentgenologic signs of pulmonary congestion on the day of the study in all patients. Four patients had left ventricular failure due to previous myocardial infarction, 1 had left ventricular failure due to acute myocardial infarction, one had arterial hypertension with an episode of acute left ventricular failure and one patient had aortic valvular insufficiency with left ventricular failure.

Five patients were on long-term treatment with digitalis and diuretic, and one patient with digitalis alone. Three of these patients received their usual dose of digitalis 4 h prior to the study, whereas the other three patients had their medication stopped on the day before the study. Two patients had a subcutaneous injection of Thebacon 6 h prior to the study. Apart from this, no medication which might influence the cardio-pulmonary function was given during the night or morning before the start of the experiment.

The patients were studied as soon as practically possible after the admission to hospital, but some degree of clinical improvement would usually already have occurred before the time of the study.

METHODS

The patients were studied in the supine position, at room temperature, breathing air. A Cournand catheter no. 7 was introduced into the pulmonary artery wedge position under direct fluoroscopic control. A polyethylene catheter was introduced into the femoral artery. Three control readings were taken approximately 10 min apart. On each occasion records were made of FAP, PCVP, PAP and ECG, and blood samples for blood gas analyses were taken from the pulmonary artery and the femoral artery. A deep intramuscular injection of 15 mg of morphine chloride was given, and the same set of recordings were made at 15-minute intervals for 60 to 75 min after the injection of morphine.

PAP and PCVP were measured with a Statham Transducer P 23 BB, and FAP with Statham Transducer P 23 GB. All pressures and their integrated means were recorded on an Elema Schönander Mingograph no. 4.

The oxygen tension (PO_2) and the carbon dioxide tension (PCO_2) were measured by means of an IL Gas Analyser Model 113, and a Radiometer pH Meter Model 27 with Micro Electrode Unit. The Hb oxygen saturation (SO_2) was read from Radiometer Blood Gas Calculator (Severinghaus 1966). The correctness of the readings was frequently checked by direct spectrophotometric determination with a Beckman Spectrophotometer Model DU according to Refsum (9). The Hb concentration was measured by the cyanmethaemoglobin method, and the O_2 content was calculated from the Hb oxygen saturation and the Hb concentration, assuming that the oxygen capacity of 1 g Hb is 1.4 ml. The base excess was read from a Siggaard-Andersen alignment nomogram (11), the average of the simultaneously taken arterial and venous samples being presented.

RESULTS

The observations are shown in Figs. 1, 2 and 3. The observations in the two patients with the most pronounced disturbances of pulmonary vascular pressure and blood gases, showing considerable fluctuations from one set of observations to another, have been marked separately and have not been included in the calculation of average values.

Before morphine

Clinical. Patient A was somewhat breathless in the supine position. Patient B, with pronounced Cheyne-Stokes respiration, was drowsy and confused. The remainder of the patients were in no distress.

ECG. One patient was in atrial fibrillation, the others in sinus rhythm. Two patients had a few ventricular extrasystoles.

Heart rate. The heart rate varied from 66 to 112 beats/min, the average being 85 beats/min.

Systemic arterial blood pressure. The mean FAP varied from 80 to 120 mmHg, with an a er age of 97 mmHg.

Pulmonary arterial blood pressure. All patients showed mean PAPs near or above the upper limit of normal for resting subjects (20 mmHg). In patients A and B it was 48 and 45 mmHg, respectively. In the rest of the patients it varied from 20 to 39 mmHg, with an average of 28 mmHg.

Pulmonary capillary venous pressure. In patients A and B the mean PCVP was markedly elevated above the upper limit of normal (12 mmHg), being 34 and 26 mmHg, respectively. In the rest of the patients the pressure was moderately elevated or normal, varying from 8 to 20 mmHg, with an average of 13 mmHg.

Blood gases. In *arterial blood* (Fig. 2) patients A and B showed considerable fluctuations from one set of observations to another with markedly reduced PCO and increased pH in the first samples. The rest of the patients showed fairly stable values, PCO_2 was within the normal range



Fig. 2. The effect of 1.5 cg morphine chloride i.m. on the arterial PCO_2, PO_2, SO_2 and pH in eight patients with clinical pulmonary congestion. Symbols as in Fig. 1.

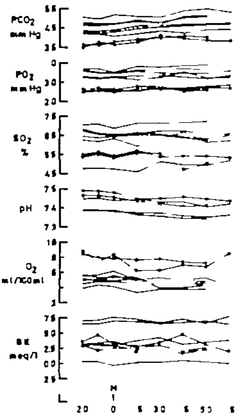


Fig. 3. The effect of 1.5 cg morphine chloride i.m. on the mixed venous PCO_2, PO_2, SO_2 and pH, and on the a–$\bar{v}O_2$ and mean base excess of the arterial and venous blood in eight patients with clinical pulmonary congestion. Symbols as in Fig. 1.

(35–45 mmHg), pH was within the upper part of the normal range (7.35–7.45) or slightly above, whereas all PO_2 and SO_2 values were more or less reduced below the lower limit of normal (75 mmHg and 95% respectively). The average values were $PaCO_2$ 40.8 mmHg, pH 7.453 PaO_2 57.9 mmHg, SaO_2 90.1%.

In *mixed venous blood* (Fig. 3) patients A and B showed very stable values; PCO_2 was clearly below normal and pH above normal, whereas PO_2 and SO_2 were markedly reduced. The rest of the patients in general showed normal PCO_2, somewhat increased pH, and clearly reduced PO_2 and SO_2, all values of SO_2 being at or below the lower limit of normal (68%). The average values were $P\bar{v}CO_2$ 45.6 mmHg, p$\bar{v}$H 7.413 $P\bar{v}O_2$ 32.9 mmHg and $S\bar{v}O_2$ 62.8%.

Patients A and B showed a marked elevation

of a-vO$_2$ above the upper limit of normal (5 ml/100 ml) whereas the others showed normal or moderately elevated values. BE was in general somewhat higher than normal. The average values were a-vO$_2$ 5.0 ml/100 ml and BE 3.34 mEq/L.

After morphine

Clinical. All patients experienced drowsiness which, however, began to wear off towards the end of the observation period. Dyspnea was relieved in patient A, while Cheyne-Stokes respiration was more pronounced after morphine in patient B. One patient complained of short-lasting nausea.

ECG. In the two patients with ventricular extrasystoles the number of ventricular extrasystoles was slightly increased.

Heart rate. In all but two patients there was a slight decrease in heart rate.

Systemic arterial blood pressure. There was a clear decrease in mean FAP in patients A and B but only a moderate tendency to fall in the rest of the patients.

Pulmonary arterial blood pressure. There was a slight tendency to decrease in the mean PAP in patients A and B, but no change was observed in the rest of the patients.

Pulmonary capillary venous pressure. In patient A mean PCVP dropped by 10 mmHg, but returned to its initial value at the end of the observation period. In the other patients there was no change.

Blood gases. In *arterial blood* (Fig.) patients A and B still showed considerable fluctuations and, although some PCO$_2$ values were above and some pH, PO$_2$ and SO$_2$ values fell below those in the control period, there were no unequivocal changes. In the rest of the patients there was a general tendency to an increase in PCO$_2$ and a decrease in pH, PO$_2$ and SO$_2$, with the maximal deviation from the control period values 60 min after the injection of morphine. At this time the average values were PaCO$_2$ 45.6 mmHg, paH 7.419, PaO$_2$ 55.4 mmHg and SaO$_2$ 87.2%.

In *mixed venous blood* (Fig. 3) patients A and B showed far less fluctuations than in the arterial blood and behaved as the rest of the patients. PCO$_2$ showed a general tendency to increase and pH a tendency to decrease with maximal deviations from the control period values 60 min after the injection of morphine. PO$_2$ and SO$_2$ remained unchanged, in contrast to the findings in the arterial blood, PO$_2$ showing a remarkable constancy throughout the investigation. The average values 60 min after the injection of morphine were PvCO$_2$ 49.9 mmHg, pvH 7.381, PvO$_2$ 33.9 mmHg and SvO$_2$ 62.7%.

In general a-vO$_2$ showed a tendency to decrease, particularly in patients A and B, whereas BE remained unchanged. The average values 60 min after the injection of morphine were a-vO$_2$ 4.67 ml/100 ml and BE 3.12 mEq/L.

DISCUSSION

The diagnosis of pulmonary congestion in the patients subjected to this study was based on the findings at clinical examination and on the chest X-ray on the day of the study. The arterial blood gas observations, showing normal or reduced PCO$_2$ and clearly reduced PO$_2$ and SO$_2$ in all the patients, fit well with pulmonary congestion. However, only two patients (patients A and B) showed clearly elevated pulmonary capillary venous pressure. In all the other patients this pressure was normal or only moderately elevated. The most probable explanation of this is that, at the time of the study, recovery from the pulmonary congestion was already under way in most patients. This is in accordance with the observations of others (1) on the process of resolution of pulmonary edema, that the pulmonary capillary venous pressure returns to normal before normalization of the chest X-ray and the alveolar-arterial gas exchange.

The findings of low PO$_2$ and SO$_2$ in mixed venous blood and of increased a-vO$_2$ prior to morphine indicate reduced cardiac output, particularly in patients A and B. This might be due to poor myocardial function at the time of the study, but might also be due to a reduced cardiac output response to hypoxia, consequent on a prolonged period with poor myocardial function prior to the investigation. The latter possibility fits well with the finding of a relatively moderate increase in PCVP and with the suggestion above that the patients were in a recovery phase. However, as shown by the BE values, neither the low cardiac output nor the arterial hypoxemia have

led to metabolic acidosis. The relatively high BE values may most probably be ascribed to the treatment with diuretics.

The increase in PAP observed in the control period prior to morphine is relatively greater than the elevation in PCVP. Since none of the patients showed evidence of primary pulmonary disease, it is likely that the high pulmonary arterial pressures reflect the presence of pulmonary changes secondary to long-standing pulmonary congestion (13). The marked fluctuations in the arterial blood gases in patients A and B throughout the study no doubt reflect true variations in these ery sick patients, but make it difficult to draw conclusions as to changes in their a erage situa tion.

The administration of morphine led to an increase in PCO_2 and a relatively somewhat smaller decrease in PO_2 in the arterial blood, indicating a definite tendency to depression of the alveolar ventilation, but no clear further impairment of the alveolar/arterial gas exchange.

It would seem quite possible that the reduction of PO_2 and SO_2 in arterial blood after morphine could lead to increased tissue hypoxia, particularly if the cardiac output were reduced further- but the fact that PO_2 in mixed venous blood remained unchanged indicates that the tissue oxygenation on the whole did not suffer provided that the distribution of arterial blood was unchanged after morphine. This is further supported by the constancy of the BE, which shows that there was no tendency to development of metabolic acidosis, the slight fall in pH in arterial and mixed venous blood being solely a consequence of the increase in PCO_2. This is in accordance with previous observations of Hoel and Refsum (3) in patients with acute myocardial infarction.

It is remarkable that PO in mixed venous blood remained essentially unchanged throughout the experiment, even in the presence of marked variations in the arterial blood gases and in the systemic arterial and pulmonary capillary enous pressures in patients A and B. This seems to indicate the presence of some O_2-dependent regula tion of the peripheral circulation on the enous side.

The slight reduction in a O after morphine is probably rather a consequence of reduced O_2 intake, as usually observed under the influence of morphine, than of increase in cardiac output.

Reduction in systemic arterial blood pressure is the predominant feature of the hemodynamic changes after morphine With presumably unchanged cardiac output, this would indicate that morphine led to a reduction in the cardiac work, particularly in the sickest patients (patients A and B) This seems to be in accordance w th the assumption based on experiments in dogs, that the beneficial effect of morphine in pulmonary con gestion is due to a pharmacological phlebot m brought about by increase in peripheral ascular capacitance w th consequent reduction in cardia output (7 8, 14).

The clear fall of the PCVP during the first half-hour after morphine in patient A indicates, together with the decrease in heart rate, that the injection of morphine was primarily accompanied by an improvement of the left entricular per formance. The interpretation of the further course is, however uncertain The rise of the PCVP might reflect increasing left entricular failure, which could be ascribed to too marked fall of the FAP and a consequent reduction of the heart perfusion, together with fall in the ar terial PO_2 and SO_2. However none f the other observations, particularly the en PO a- O_2, BE and heart rate, as well as the linical in vestigation after the study ga e e idence of im pairment of the heart performance

In summary this study has shown that an ordinary dose of morphine given to patients with pulmonary congestion causes a slight depression of alveolar ventilation. Howe er the use of morphine does not seem to be followed by impair ment of tissue oxygenation and there is some evidence that it may lead to reduction the car diac work. According to these data we find no reason to ad ocate that the use of morphine should be discontinued in this type of patients.

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Acta med. scand. Vol. 190 pp. 555–559 1971

THE DIFFERENCE BETWEEN THE PULMONARY ARTERY DIASTOLIC PRESSURE AND THE PULMONARY WEDGE PRESSURE IN CHRONIC LUNG DISEASE

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Abstract. Twenty-four patients with chronic lung disease have been examined by means of right heart catheterization to investigate the level of the pressure difference between the pulmonary artery diastolic pressure and the mean pulmonary artery wedge pressure ($P_{PA_d} - \bar{P}_W$) and its relation to the pulmonary vascular resistance at rest and during exercise. In patients who had a normal pulmonary vascular resistance (PVR ≤ 3 units), there was no significant pressure difference either at rest or during exercise when $\bar{P}_W$ was 6–27 mmHg, while in patients with increased pulmonary resistance (PVR > 3 units) a significant pressure difference has been found. A pressure difference between 4 and 5 mmHg was found, in the present material, to be an index for differentiation between a normal and an increased vascular resistance.

Increasing interest has been focused recently on the study of the pressure gradient across the pulmonary vascular bed during diastole. This is defined as the difference between the pulmonary artery diastolic pressure (P_{A_d}) and the venous diastolic pressure. Kaltman et al. (12) found in patients with heart disease that, in the absence of pulmonary vascular or mitral valvular disease, the pulmonary artery diastolic pressure is identical to, and increases linearly with, the left ventricular end diastolic pressure (LVED). Sapru et al. (17) showed that the mean pulmonary artery wedge pressure ($\bar{P}_W$) is an accurate measure of LVED both at rest and during exercise. It seems likely therefore, that there should be no significant pressure difference between P_{PA_d} and $\bar{P}_W$, a fact that has been shown by Harvey et al. (10) in patients with Asiatic cholera and without discernible heart or lung disease. Cournand (3) has suggested that a rough qualitative evaluation of functional or anatomical sources of pulmonary vascular resistance should be based on the magnitude of the diastolic pressure gradient (P_{PA_d}–LVED).

This study was undertaken to investigate the level of the pressure difference between the pulmonary artery diastolic pressure and mean pulmonary artery wedge pressure (P_{PA_d}–$\bar{P}_W$) and its relation to the pulmonary vascular resistance (PVR) at rest and during exercise in patients with chronic lung disease.

MATERIAL AND METHODS

The results are derived from the examination of 24 patients with chronic lung disease who had undergone a functional study using right heart catheterization. None of the patients had any evidence of congestive heart failure or valvular disease. The patients were classified into two groups according to their PVR. Group I consisted of 12 patients, 7 men and 5 women, aged 33–64 years (average 51.3) and having PVR ≤ 3 units, group II of 12 patients, 8 men and 4 women, aged 49–66 years (mean age 57.7) and having PVR > 3 units. In this laboratory the normal value for PVR index = 0.6–3.0 units (11).

Half of the patients in each group had chronic obstructive disease of bronchitis-emphysema type, the rest had restrictive lung disease. All patients were examined by means of right heart catheterization in the recumbent position at rest and during exercise with a cycle ergometer. The technique used in this laboratory has been described earlier (1). The average work load in group I was 275 kpm/min and in group II 200 kpm/min. P_{PA_d} and $\bar{P}_W$ were recorded simultaneously with a double lumen catheter in all except three patients in whom a single lumen catheter was used. During exercise the pressures were recorded after 2 min from the start of work. The cardiac output (C.O.) was determined according to the direct Fick method between 4–5 min exercise. PVR index was calculated according to the equation ($\bar{P}_{PA}$–$\bar{P}_W$)/cardiac index. The heart rate (HR) was that obtained during the measurement of P_{PA_d} and $\bar{P}_W$, and in the three patients in whom a single lumen catheter was used the HR was calculated as the mean of two values.

RESULTS

Haemodynamic data are shown in Table I. In group I with normal PVR, $\bar{P}_W$ at rest averaged

Table I. *Haemodynamic data at rest (R) and during exercise (E) in groups I and II*

Mean values ± S.D., range in parentheses

	P_{PA_d}	Pw	P_{PA_d}-Pw	PVR	CO	HR	SaO_2
Group I (=12)							
R	10._ 1 8 (8-13)	9 6 _2 (6-15)	0 6 _ 2 0 (-2 4)	2.1 _ 0.5 (1 5-3)	6 1 ± 1 1 (4 6-7.3)	80.5 ± 12.4 (66-114)	94.1 _ 1 9 (90.9-97.8)
E	18.7 5 0 (12 ?7)	17.2 6 0 (8 27)	15 _ 1 7 (-3- 4)	2.3 _ 0 2 (2 0-2 8)	10.1 ± 2.3 (6 4-13 9)	114 3 15.6 (89 154)	92.8 ± 3 2[a] (85.7 96.9)
Group II (=1_)							
R	14.5 _ 5 4 (5-?8)	7 9 _ 3 9 (0-15)	6.6 2 3 (5-13)	4 6 _ 1.0 (3 6-7 1)	5 4 0 7 (4.1 6 8)	77 1 _ 9.5 (59-86)	93 9 ± 3.1 (86.3-98.4)
E	_6 1 ± 8.4 (13-48)	13 9 6.3 (4- 4)	1_ 5 6 (7 6)	5.3 _ 1 4 (3 8 1)	8 8 1 7 (6 8 1 0)	101 9 _ 13 1 (78 119)	89 4 _ 7 4 (7 6-96.2)
p I II							
R	0 05	0 05	<0 001	<0 001	0 05	0 05	0 05
E	0 05	0 05	<0 001	<0 001	0 05	0 05	0.05

No. of observations = 11

9 6 mmHg, S D ± — 11 patients had a pr s sure equal to or less than 11 mmHg, one patient had 15 mmHg. The values ere within the same range as in normal material studied with the same technique (11). During e ercise Pw in creased significantly and averaged 17 2 mmHg S D ± 6 0. All indi idual pre ures increased sig nificantly except in two patients in whom the increase was 1 and mmHg In fi e patients whose pressures at r t re 8 8 9 10 and 11 mmHg, their pressu se during exercise to 20, 27 _2 17 and m pe t vely The mean Pw during exer c h gher than that re ported n normal differ ne ertheless from that found ld men (18) How ever the rise of individual pressure was higher than reported in normal subjects (4 11).

P_{PA_d} at rest averaged 10.2 mmHg S.D. ±1.2 and did not differ from P̄w ($p > 0.05$). During exercise it increased linearly with P̄w and was highly correlated with it (r 0.965 $p < 0.001$) (Fig. 1) The increase in P_{PA_d} was proportional to the increase in Pw The variation of P_{PA_d} with Pw is described by the following regression equations.

Rest $P_{PA_d} = 6.183$ 0.416 P̄w (S.D ±1.618, r 0.516, $p > 0.05$) (1)

Exercise: $P_{PA_d} = 4.953 + 0.600$ P̄w (S.D ±1.371, r 0.965 $p < 0.001$) (2)

Rest Exercise: P_{A_d} 2.209 + 0.913 P̄w (S.D _1 895 r 0.946, p 0.001) (3)

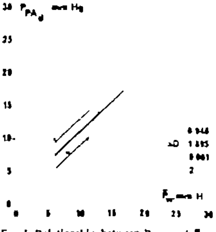


Fig 1 Relationship between P_{PA_d} nd P̄w at est (○), and during exercise (●) in group I

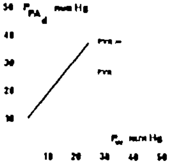


Fig 2. Regression line for the relation between P_{PA_d} P̄w t rest and during exercise in group II (—) c pared to the regression line in Fig. 1 ( ).

In group II with increased PVR, $\bar{P}w$ at rest and during exercise was not significantly different from group I ($p>0.05$), but P_{PA_d} was higher ($p<0.05$). During exercise the change in $\bar{P}w$ was approximately of the same magnitude as in group I, hile the change in P_{PA_d} was steeper and the magnitude of the change nearly twice that of $\bar{P}w$ (Fig. 2).

The pressure difference ($P_{PA_d}-\bar{P}w$)

In group I the pressure difference was small and averaged at rest 0.6 mmHg S.D. ± 2.0 ($p>0.05$) during exercise it increased slightly but not significantly ($p>0.05$) and averaged 1.5 mmHg, S.D. ± 1.7. There was a negative correlation between the pressure difference and $\bar{P}w$ at rest ($r=-0.646$, $p<0.05$) and during exercise ($r=-0.678$, $p<0.05$). It varied between 4 mmHg and -3.0 mmHg when $\bar{P}w$ was 6–27 mmHg, while a pressure difference of 5 mmHg or more was observed when $\bar{P}w$ was less than 5 mmHg in three other patients who were not included in this material. The pressure difference did not change with the cardiac output (Fig. 3 *a*). It was not correlated with PVR, HR or oxygen unsaturation.

In group II the pressure difference was significantly higher than in group I ($p<0.001$) and increased significantly during exercise ($p<0.001$). It varied irregularly with increasing cardiac output (Fig. 3 *b*) and was not correlated with it. The only significant correlation found was with PVR during exercise ($r=0.573$, $p<0.05$). The lowest value in this group was 5 mmHg and the highest 26 mmHg. When the pressure difference was plotted against the pulmonary vascular resistance at rest and during exercise for the whole material, a pressure level between 4 and 5 mmHg separated the two groups (Fig. 4).

Cardiac output, heart rate and oxygen saturation

There was no difference of significance between these variables in the two groups except for slightly higher heart rate during exercise in group II.

DISCUSSION

Haemodynamic studies in normal subjects have shown a close agreement between the pulmonary artery diastolic pressure and the mean pulmonary wedge pressure at rest and during exercise (5, 7). The present study similarly shows in patients with chronic lung disease and having normal pulmonary vascular resistance (group I) a close relationship between the two pressures both at rest and during exercise, when the $\bar{P}w$ was 6–27 mmHg. This close relationship resulted in a small and insignificant diastolic pressure gradient which decreased with increasing wedge pressure, as shown by the negative correlation between the pressure difference and wedge pressure, and increased when the wedge pressure was less than 5 mmHg. In patients with congenital heart disease Kaltman et al. (12) have shown a small diastolic pressure gradient, which increased when LVED was less than 3 mmHg. The close relationship between the pulmonary artery diastolic pressure and the wedge pressure (equa-

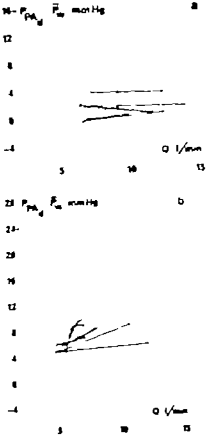


Fig. 3. The pressure difference in relation to cardiac output at rest and during exercise in group I (*a*) and group II (*b*). Symbols as in Fig. 1.

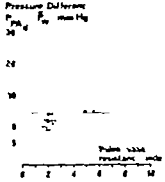


Fig. 4. The pressure difference between P_{PA_d} and $\bar{P}_W$ has been plotted against the PVR index at rest and during exercise in the whole material (n = 4). A pressure difference between 4-5 mmHg separated group I (*lower left*) from group II (*upper right*). Symbols as in Fig. 1.

tions 1-3) allows the estimation of the first from the second. Since $\bar{P}w$ is in close agreement with the left atrial pressure (6) and the latter is almost identical with LVED (2) the wedge pressure would reflect the left ventricular end diastolic pressure, a fact that has been shown by Malmborg (16) Sapru et al. (17) and Lockhart et al. (14). The P_{PA_d} can thus be used as an index for the filling pressure in the left ventricle in patients with normal PVR and with a normal mitral valve.

When the patients had an abnormal pulmonary vascular bed and increased PVR (group II) a significant pressure difference existed between P_{A_d} and Pw and in such conditions the P_{A_d} cannot be used as index for LVED. At rest the pressure difference was caused by the elevation of P_{PA_d} as Pw was normal. During exercise the increment in $\bar{P}w$ was 6 mmHg, while P_{PA_d} increased 1 mmHg. This shows that the further widening of the pressure difference observed during exercise was also caused by the independent change of P_{PA_d}, and the rise in Pw was not responsible for it. The large pressure difference is not attributed to the change in the blood flow as the cardiac output was lower in group II than in group I both at rest and during exercise. Some investigators found a significant correlation between the oxygen unsaturation and the diastolic pressure gradient (10, 14) an observation that was not confirmed in this study. However Wranne (19) has shown in patients with pulmonary tumours, who were submitted to lung surgery that the oxygen tension decreased and PVR increased after operation and that there was a significant pressure difference between the pulmonary artery diastolic pressure and left atrial pressure during air breathing, which did not decrease with oxygen breathing. Harvey et al. (9) found that induced acidosis was responsible for the diastolic pressure gradient in patients with obstructive lung disease, a factor that was not studied here. The probability that altered distensibility of the large elastic arteries might be a cause of the increase of pressure difference has been disproved by Harvey et al. (10). Comparison between the haemodynamic variables in the two groups (Table 1) shows that PVR was the only variable that was significantly different, to which the large pressure difference in group II could be ascribed.

It would appear that an increase in PVR is the final result of oxygen unsaturation, acidosis or reduction in the size of the pulmonary vascular bed, factors which, separately or combined, are features of chronic lung disease (14).

The contribution of PVR to the level of pressure difference was studied. The highest value reported in group I was 4 mmHg, while 5 mmHg was the lowest value in group II. Thus a pressure difference between 4 and 5 mmHg seems likely to be an index for differentiation between normal and abnormal PVR (Fig. 4). Whether this level has a general application is debatable as patients with chronic lung disease have abnormally wide ventilatory swings of pressures and increased intrathoracic pressure, factors which cause rises in the vascular pressures and pulmonary vascular resistance (8, 13).

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Hypertensin Mechanisms. Edited by Ralph Reader D.Phil. (Oxon). 289 pp. $6.00. American Heart Association Monograph No. 3., Suppl. II to Circulation Research 26 and 27 1971

This monograph consists of 28 papers presented at an International Conference on Hypertension Mechanisms organized by National Heart Foundation of Australia in Jan. 1970.

A broad outlook on modern thought and recent progress in this vast and highly important field of medical investigation is presented. T main streams of interest can be discerned among the authors, the autonomic nervous system and the renal adrenal control.

Modern techniques for investigation of autonomic function used in most of the nine papers dealing with this subject g much new information, but the important question of cause or effect still remains unsolved.

The monograph present a comprehensive view of the field of renal-adrenal control mechanisms in 11 papers. Much new information is here easily accessible though full agreement about the importance of the renin-angiotensin-aldosterone system does not exist between all investigators.

Of particular interest is the angiotensin effect demonstrated on the autonomic nervous system and the renin release caused by norepinephrine. Thus points of contact has been shown between neurogenic and renal hypertension.

The data presented on renal effect of hydralazine treatment are also of interest. Circulatory physiology, the sodium problem and physical properties of the arterial wall are other aspects considered. The book is strongly recommended to all colleagues interested in obtaining an extensive view of modern research in the field of hypertensive mechanisms.

P. O. Wester, Stockholm

Local Regulation of Blood Flow. Edited by Simon Rodbard, M.D. 158 pp. $6.00. American Heart Association Monograph No. 33. 1971. Suppl. I to Circulation Research 28 and 29. 1971.

This monograph is a summary of the information presented at an International Symposium on the Local Regulation of Blood Flow at the City of Hope, Duarte, Calif., USA, in Jan. 1970, and reports on new information and concepts concerning this key aspect of physiology and medicine.

The resistance vessels of the tissues show the remarkable characteristic of being able to regulate the delivery of blood to each tissue so efficiently that local requirements are met without deficiency or excess blood flow. This appears to be accomplished primarily by local mechanisms, most of which are as yet incompletely understood. This monograph presents a clear and comprehensive survey of the current front line in this field of research. Most of the papers are concerned with the regulation of blood flow in skeletal muscle and myocardium. Of particular interest is the discussion of the role of tissue hyperosmolality in exercise hyperemia.

Clarification of the mechanisms underlying the local regulation of blood flow may be expected to improve the ability of the physician to treat myocardial ischemia, to control hypertension, to reverse the process of vascular shock, etc. This monograph thus provides valuable and easily accessible information in a field of physiology which should interest a wide circle of readers besides those in physiological laboratories.

John Wahren, Stockholm

Congress Announcement

Symposium on Clinical Aspects of Metabolic Bone Disease will be held at Henry Ford Hospital, Detroit, Michigan, June 26–28, 1972. Invited speakers. Ten-minute papers on the clinical aspects of metabolic bone disease will also be accepted for consideration until 3 1 72.

Information: Dr Boy Frame, Henry Ford Hospital, Detroit, Michigan 48202, USA.

SUPPLEMENTS TO VOLUME 190

SUBJECT INDEX

(Supplements, see page V)

Diabetes mellitus

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